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# ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS



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## Tuesday—Afternoon Session

CONTRIBUTED PAPERS



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# PROCEEDINGS OF THE SIXTY-FIFTH ANNUAL MEETING OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1951

The sixty-fifth annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., October 1, 2, and 3, 1951.

The meeting was called to order by the President, H. A. Halvorson, on the morning of October 1, at 10:00 o'clock.

## OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER 1952

### President

H. A. Halvorson, U. S. Food and Drug Administration, Washington, D. C.

### Vice-President

H. J. Fisher, Conn. Agricultural Experiment Station, New Haven, Conn.

### Secretary-Treasurer Emeritus

W. W. Skinner, Kensington, Md.

### Secretary-Treasurer

Kenneth L. Milstead, U. S. Food and Drug Administration, Washington 25, D. C.

### Additional Members of the Executive Committee

E. L. Griffin, Washington, D. C.
W. F. Reindollar, Baltimore, Md.
K. D. Jacob, Beltsville, Md.
H. A. Halvorson, St. Paul, Minn.

### Editorial Board

Kenneth L. Milstead, *General Chairman*

*Editorial Committee of The Journal*

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

E. L. Griffin (1952) — J. B. Smith (1953)
F. H. Wiley (1952) — A. H. Robertson (1955)
Katharine Ronsaville, *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 — J. O. Clarke

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

## 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:

E. L. GRIFFIN (1952), (Production and Marketing Adm., Washington 25, D. C.), *Chairman;* J. B. SMITH (1954); and C. V. MARSHALL (1956).

FEEDING STUFFS:

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

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

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

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

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

TANKAGE (HIDE, HOOF, HORN, AND HAIR CONTENT):
A. T. Perkins, Kansas State College, Manhattan, Kans.

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

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

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

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

FERTILIZERS:

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

SAMPLING AND PREPARATION OF SAMPLE:
Stacey B. Randle, N. J. Agr. Expt. Station, New Brunswick, N. J.

PHOSPHORIC ACID:
K. D. Jacob, Plant Industry Station, Beltsville, Md.

FREE WATER:
W. L. Hill, Plant Industry Station, Beltsville, Md.

NITROGEN:
H. A. Davis, Agricultural Experiment Station, Durham, N. H.

MAGNESIUM AND MANGANESE:
John B. Smith, Agricultural Experiment Station, Kingston, R. I.

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

POTASH:
O. W. Ford, Purdue University, Lafayette, Ind.

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

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† 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.
BORON:
Rodney Berry, Va. Department of Agriculture, Richmond, Va.
INERT MATERIALS:
K. G. Clarke, Division of Fertilizer and Agricultural Lime, Beltsville, Md.

ECONOMIC POISONS:

*Referee:* J. J. T. Graham, Production and Marketing Administration, Insecticide Division, Washington 25, D. C.
2,4-DICHLOROPHENOXYACETIC ACID AND RELATED COMPOUNDS:
A. B. Heagy, Md. Inspection and Regulatory Service, College Park, Md.
ISOPROPYL PHENYL CARBAMATES AND RELATED COMPOUNDS:
Roy Shaw, State Department of Agriculture, Salem, Oreg.
RODENTICIDES:
J. B. LaClair, Department of Agriculture, Sacramento 14, Calif.
BENZENEHEXACHLORIDE:
Irwin Hornstein, Bur. Entomology and Plant Quarantine, Beltsville, Md.
ORGANIC THIOCYANATES:
H. A. Rooney, Department of Agriculture, Sacramento 14, Calif.
DIMETHYL DITHIOCARBAMATES:
J. D. Patterson, Department of Agriculture, Salem, Oreg.
DDT AND RELATED COMPOUNDS:
E. E. Fleck, Bur. Entomology and Plant Quarantine, Beltsville, Md.
PARATHION:
F. I. Edwards, Bur. Entomology and Plant Quarantine, Beltsville, Md.
CHLORDANE AND TOXAPHENE:
T. H. Harris, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
PYRETHRINS:
D. Kelsey, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
PIPERONYL BUTOXIDE:
B. L. Samuel, Div. Chemistry, Dept. of Agriculture, Richmond 19, Va.
ROTENONE:
R. Payfer, Plant Products Div., Dept. of Agriculture, Ottawa, Canada
ALLETHRIN:
M. S. Konecky, Bur. Entomology and Plant Quarantine, Beltsville, Md.
QUATERNARY AMMONIUM COMPOUNDS:
R. L. Caswell, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
PHENOLIC DISINFECTANTS:
W. A. Affens, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
PHYSICAL PROPERTIES OF ECONOMIC POISONS:
E. L. Gooden, Bur. Entomology and Plant Quarantine, Beltsville, Md.
ALDRIN:
S. J. Few, Miss. State Chem. Laboratory, State College, Miss.
DIELDRIN:
J. B. McDevitt, Jr., Feed and Fertilizer Lab., Louisiana State College, Baton Rouge 3, La.

DISINFECTANTS:

*Referee:* L. S. Stuart, Production and Marketing Adm., Livestock Branch, Washington 25, D. C.

MEDIA FOR DISINFECTANT TESTING:

Michael J. Pelzar, Dept. of Bacteriology, Univ. of Md., College Park, Md.

FUNGICIDES AND SUBCULTURE MEDIA:

L. F. Ortenzio, Agricultural Research Center, Beltsville, Md.

PLANTS:

*Referee:* E. J. Miller, Agricultural Experiment Station, East Lansing, Mich.

SAMPLING:

E. J. Miller

BORON:

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

SUGAR:

Kenneth T. Williams, Western Regional Research Lab., Albany, Calif.

ZINC:

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.

CAROTENE:

E. J. Benne

SODIUM:

Eunice J. Heinen, Mich. Agricultural Experiment Sta., East Lansing, Mich.

STARCH:

Carroll L. Hoffpauir, Southern Regional Research Lab., New Orleans, La.

SOILS AND LIMING MATERIALS:

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

HYDROGEN-ION CONCENTRATION OF SOILS:

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

BORON:

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

FLUORINE:

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

ZINC AND COPPER:

W. L. Lott, U. S. Bur. Plant Industry, Soils, and Agri. Eng., Raleigh, N. C.

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.

PHOSPHORUS:

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

MOLYBDENUM:

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

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.

VITAMINS:

*Referee:* Chester D. Tolle, Food and Drug Administration, Washington 25, D. C

VITAMIN A IN OLEOMARGARINE:

J. B. Wilkie, Food and Drug Administration, Washington 25, D. C.

VITAMIN A IN FEEDS:

Maxwell L. Cooley, General Mills, Inc., Larrowe Div., Rossford, Ohio

VITAMIN $B_6$ (CHEMICAL):

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

VITAMIN D—POULTRY FEED SUPPLEMENTS:

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

NICOTINIC ACID (CHEMICAL):

J. P. Sweeney, 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.

VITAMIN $B_{12}$ (MICROBIOLOGICAL METHOD):

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

THIAMINE IN ENRICHED CEREAL PRODUCTS:

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

SUBCOMMITTEE B:

F. H. Wiley (1952), (Food and Drug Administration, Washington 25, D. C.) *Chairman;* HARRY J. FISHER (1954); and G. R. CLARK (1956).

RADIOACTIVITY:

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

SPECTROGRAPHIC METHODS

*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.

RUTIN IN TABLETS:

A. Turner, Eastern Regional Research Lab., U. S. Department of Agriculture, Philadelphia, Pa.

SYNTHETIC DRUGS:

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

METHYLENE BLUE:

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

SULFANILAMIDE DERIVATIVES:

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

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.

DI- AND TRIPHENHYDRAMINE HYDROCHLORIDE (BENADRYL ® AND PYRIBENZAMINE ®):

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

SYNTHETIC ESTROGENS:

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

AMPHETAMINE AND DEXEDRINE SULFATES:

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

MISCELLANEOUS DRUGS:

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

MICROSCOPIC TESTS FOR ALKALOIDS AND SYNTHETICS:

W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.

MERCURY COMPOUNDS:

R. D. Herd, Food and Drug Administration, St. Louis 1, Mo.

ORGANIC IODIDES AND SEPARATION OF HALOGENS:

V. E. Stewart, State Department of Agriculture, Tallahassee, Fla.

ALKALI METALS:

J. E. Callison, Food and Drug Administration, Chicago 7, Ill.

GLYCOLS AND RELATED COMPOUNDS:

Harry Isacoff, Food and Drug Administration, New York 14, N. Y.

PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS:

C. N. Jones, Food and Drug Administration, New York 14, N. Y.

COSMETICS:

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

COLD PERMANENT WAVES:

Henry Kramer, Food and Drug Administration, Washington 25, D. C.

COSMETIC CREAMS:

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

DEODORANTS AND ANTI-PERSPIRANTS:

Henry Kramer

HAIR DYES AND RINSES:

S. W. Newburger, Food and Drug Administration, Washington 25, D. C.

MASCARA, EYEBROW PENCILS, AND EYE SHADOW:

Paul W. Jewel, Max Factor and Company, Hollywood, Calif.

SUN TAN PREPARATIONS:

S. W. Newburger

COAL-TAR COLORS:

*Referee:* K. A. Freeman, Food and Drug Administration, Washington 25, D. C.
INTERMEDIATES IN TRIPHENYL-METHANE DYES:
A. T. Schram, National Aniline Division, P. O. Box 975, Buffalo 5, N. Y.
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.
IDENTIFICATION OF COAL-TAR COLORS:
C. Graichen, Food and Drug Administration, Washington 25, D. C.
VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS:
K. S. Heine, Jr., Food and Drug Administration, Washington 25, D. C.
NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
K. S. Heine, Jr., 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.
UNSULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:
H. Holtzman, Ansbacher-Siegle Corp., Rosebank, Staten Island, N. Y.
SULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:
John Walton, H. Kohnstamm & Company, Brooklyn 31, N. Y.
INTERMEDIATES DERIVED FROM PHTHALIC ACID:
C. Graichen
LAKES AND PIGMENTS:
C. Graichen
SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS:
J. H. Jones
SUBSIDIARY DYES IN D&C COLORS:
L. Koch, H. Kohnstamm and Company, Brooklyn 31, N. Y.
HEAVY METALS IN COAL-TAR COLORS:
C. Stein, 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.
SUBSIDIARY DYES IN FD&C COLORS:
M. Dolinsky, Food and Drug Administration, Washington 25, D. C.
BOILING RANGE OF AMINES DERIVED FROM COAL-TAR COLORS:
L. S. Harrow
PAPER CHROMATOGRAPHY OF COAL-TAR COLORS:
Doris Tilden, Food and Drug Administration, San Francisco, Calif.
INORGANIC SALTS 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. (1952).

PROCESSED VEGETABLE PRODUCTS:

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

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.

PHOSPHATASE TEST IN DAIRY PRODUCTS:

L. H. Burgwald, Ohio State University, Columbus, Ohio

SAMPLING, FAT, AND MOISTURE IN HARD CHEESES:

Wm. Horwitz

PREPARATION OF BUTTER SAMPLES:

A. L. Weber, Food and Drug Administration, New York 14, N. Y.

TESTS FOR RECONSTITUTED MILK:

George F. Edwards, Mass. Dept. of Health, State House, Boston, Mass.

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.

SAMPLING, AND PREPARATION OF SAMPLE, OF SOFT CHEESES:

Sam Perlmutter, Food and Drug Administration, Minneapolis, Minn.

FROZEN DESSERTS:

H. M. Boggs, Food and Drug Administration, Philadelphia 6, Pa.

CRYOSCOPY OF MILK:

---

EGGS AND EGG PRODUCTS:

*Referee:* F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio

ADDED GLYCEROL:

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

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.

FISH PRODUCTS (ACIDS):

Fred Hillig, Food and Drug Administration, Washington 25, D. C.

SHELLFISH:

George McClellan, Food and Drug Administration, New Orleans 16, La.

ANIMAL FECAL MATTER:

John Thomas, Food and Drug Administration, New Orleans 16, La.

PINEAPPLE (DECOMPOSITION, CARBOHYDRATE):

J. F. Weeks, Jr., Food and Drug Administration, New Orleans 16, La.

APPLES (GALACTURONIC ACID):

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

STRAWBERRIES (PIGMENTS):

H. P. Bennett, Food and Drug Administration, New Orleans 16, La.

STRAWBERRIES (GALACTURONIC ACID):

P. A. Mills, Food and Drug Administration, San Francisco 2, Calif.

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 FRUIT PRODUCTS:
Doris Tilden, Food and Drug Administration, San Francisco 2, Calif.
URIC ACID IN NUTS:
H. M. Bollinger, Food and Drug Administration, Los Angeles 15, Calif.
FISH (HISTAMINE):
D. W. Williams, Food and Drug Administration, San Francisco 2, Calif.
LACTIC ACID:

---

SPINACH (GALACTURONIC AND SUCCINIC ACIDS):
H. D. Silverberg, Food and Drug Administration, St. Louis 1, Mo.

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.
TOTAL SOLIDS IN FISH AND MARINE PRODUCTS:
Menno D. Voth
SALT AND SOLIDS IN OYSTERS:
D. D. Price, Food and Drug Administration, Baltimore 2, Md.

GUMS IN FOODS:

*Referee:* M. J. Gnagy, Food and Drug Administration, Los Angeles 15, Calif.
CHEESE (ALGINATES):
M. J. Gnagy
FROZEN DESSERTS:
Shirley M. Walden, Food and Drug Administration, Baltimore 2, Md.
CACAO PRODUCTS:
Flora G. Mendelsohn, Food and Drug Administration, Los Angeles 15, Calif.
CATSUP AND RELATED TOMATO PRODUCTS:
T. E. Strange, Food and Drug Administration, Portland, Oreg.
DRESSINGS FOR FOODS:
M. J. Gnagy

MEAT AND MEAT PRODUCTS:

*Referee:* Roger M. Mehurin, Meat Inspection Div., Bur. of Animal Industry, Washington 25, D. C.
MOISTURE AND FAT IN MEAT PRODUCTS:
Ernest Windham, Vet. Division, Walter Reed Army Medical Center, Washington 12, D. C.
CREATIN IN MEAT PRODUCTS:
J. M. McCoy, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.
HORSEMEAT IN GROUND MEAT (CHEMICAL):
C. E. Hynds, State Food Laboratory, Albany 1, N. Y.
HORSEMEAT IN GROUND MEAT (SEROLOGICAL):

---

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

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS:

*Referee:* P. A. Clifford, Food and Drug Administration, Washington 25, D. C.

COPPER:

A. L. Brandon, Anheuser-Busch Company, Inc., St. Louis, Mo.

ZINC:

O. R. Alexander, American Can Company, Maywood, Ill.

MERCURY:

A. K. Klein, Food and Drug Administration, Washington 25, D. C.

DDT AS SPRAY RESIDUE ON FOODS:

R. H. Carter, Bureau of Entomology and Plant Quarantine, Beltsville, Md.

INSECTICIDES IN CANNED FOODS:

E. T. Abeling, Beechnut Packing Co., Canajoharie, N. Y.

PARATHION:

P. A. Clifford

SODIUM FLUOROACETATE (1080):

L. L. Ramsey, Food and Drug Administration, Washington 25, D. C.

METHOXYCHLOR:

Frieda M. Kunze, Food and Drug Administration, Washington 25, D. C.

BENZENE HEXACHLORIDE:

A. K. Klein

FLUORINE:

P. A. Clifford

MICROBIOLOGICAL METHODS:

*Referee:* G. G. Slocum, Food and Drug Administration, Washington 25, D. C.

CANNED MEATS:

M. L. Laing, Armour & Company, Chicago 9, Ill.

CANNED ACID FOODS:

A. P. Dunningan, Food and Drug Administration, Washington 25, D. C.

CANNED VEGETABLES:

C. W. Bohrer, National Canners Assn., Washington 6, D. C.

EGGS AND EGG PRODUCTS:

M. T. Bartram, Food and Drug Administration, Washington 25, D. C.

NUTS AND NUT PRODUCTS:

William R. North, Food and Drug Administration, Washington 25, D. C.

FROZEN FRUITS AND VEGETABLES:

H. E. Goresline, Production and Marketing Administration, Poultry Division, Washington 25, D. C.

SUGAR:

E. J. Cameron, National Canners Assn., Washington 6, D. C.

CANNED FISHERY PRODUCTS:

L. R. Shelton, Jr., Food and Drug Administration, Washington 25, D. C.

MICROCHEMICAL METHODS:

*Referee:* C. O. Willits, Eastern Regional Research Lab., Philadelphia 18, Pa.

NITROGEN AND SULFUR:

C. L. Ogg, Eastern Regional Research Laboratory, Philadelphia 18, Pa.

CHLORINE AND BROMINE:

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

NUTS AND NUT PRODUCTS:

*Referee:* A. M. Henry, Food and Drug Administration, Atlanta 3, Ga.
SHREDDED COCONUT (GLYCOLS AND GLYCEROL):
A. J. Shingler, Food and Drug Administration, New York 14, N. Y.
FREE FATTY ACIDS:

---

OILS, FATS, AND WAXES:

*Referee:* Gardner Kirsten, Food and Drug Administration, New York 14, N. Y.
SPECTROPHOTOMETRIC METHODS:
David Firestone, Food and Drug Administration, New York 14, N. Y.
PEANUT OIL:
A. B. Karasz, Dept. Agriculture and Markets, Albany 1, N. Y.
ANTIOXIDANTS:
S. Kahan, 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.
VINEGAR:
G. A. Michael, Dept. Public Health, State House, Boston 33, Mass.
VOLATILE OIL IN SPICES:
N. Aubrey Carson, Food and Drug Administration, St. Louis 1, Mo.
SUGAR, ASH, AND PUNGENT PRINCIPLES IN MUSTARDS:
Jesse E. Roe, Food and Drug Administration, Denver 2, Colo.
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:
Felice A. Rotandaro, Food and Drug Administration, Philadelphia 6, Pa.

ENZYMES:

*Referee:* J. W. Cook, Food and Drug Administration, San Francisco 2, Calif.
HYDROCYANIC ACID GLUCOSIDES:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C.

SUBCOMMITTEE D:

J. WALTER SALE (1952), (Food and Drug Administration, Washington 25, D. C.), *Chairman;* FLOYD ROBERTS (1954); and KENNETH L. MILSTEAD (1956).

ALCOHOLIC BEVERAGES:

*Referee:* J. Walter Sale, Food and Drug Administration, Washington 25, D. C.

MALT BEVERAGES, SIRUPS, EXTRACTS, AND BREWING MATERIALS:
Robert I. Tenney, Wahl-Henius Inst., 64 E. Lake St., Chicago, Ill.

HOPS:
F. E. Connery, P. Ballentine & Sons, 57 Freeman St., Newark 1, N. J.

INORGANIC ELEMENTS IN BEER:
A. L. Brandon, Anheuser-Busch, Inc., St. Louis, Mo.

COLOR AND TURBIDITY IN BEER:
B. L. Scallet, Research Division, Anheuser-Busch, Inc., St. Louis, Mo.

DISTILLED SPIRITS:
A. D. Etienne, Bureau of Internal Revenue, Washington 25, D. C.

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

NON-VOLATILE ACIDS IN WINES BY CHROMATOGRAPHY:
Alex P. Mathers

PHOSPHATES IN WINES AND SPIRITS:
M. J. Pro, Bur. Internal Revenue, Washington 25, D. C.

TANNIN IN WHISKIES AND WINES:
M. J. Pro

WINES:
Peter Valaer, Bur. Internal Revenue, Washington 25, D. C.

CORDIALS AND LIQUEURS:
John B. Wilson, Food and Drug Administration, Washington 25, D. C.

METHANOL:
J. F. Guymon, Agr. Expt. Station, College of Agriculture, Davis, Calif.

CACAO PRODUCTS:

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

LECITHIN:
J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.

MALT SOLIDS:
E. W. Meyers, Hershey Chocolate Company, Hershey, Pa.

PECTIC ACID:
H. Shuman, Food and Drug Administration, Philadelphia, Pa.

CACAO INGREDIENTS:
W. O. Winkler

LACTOSE:
Donald G. Mitchell, Walter Baker Co., Dorchester 24, Mass.

EREAL FOODS:

*Referee:* V. E. Munsey, Food and Drug Administration, Washington 25, D. C.

STARCH IN RAW AND COOKED CEREALS:
F. H. Collins, Food and Drug Administration, Cincinnati, Ohio

MILK SOLIDS AND BUTTERFAT IN BREAD:
V. E. Munsey

SOYBEAN FLOUR:
T. C. Law, 136 Forrest Ave., Atlanta, Ga.

BAKED PRODUCTS (SUGARS)
R. P. Smith, National Biscuit Co., 449 W. 14th St., New York, N. Y.

MOISTURE:
H. B. Dixon, Grain Branch, Prod. & Marketing Administration, Washington 25, D. C.

BROMATES IN FLOUR:
W. L. Rainey, Commander-Larabee Milling Co., Minneapolis, Minn.
UNSAPONIFIABLE MATTER AND STEROLS IN NOODLES AND BAKERY PRODUCTS:
V. E. Munsey
ALBUMEN IN NOODLES AND MACARONI PRODUCTS:
V. E. Munsey
MOLD INHIBITORS:
L. H. McRoberts, Food and Drug Administration, San Francisco 2, Calif.
YEAST:
A. L. Brandon, Research Div., Anheuser-Busch, Inc., St. Louis, Mo.

BAKING POWDERS AND BAKING CHEMICALS:

*Referee:* V. E. Munsey, Food and Drug Administration, Washington 25, D. C.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS:

*Referee:* K. L. Harris, Food and Drug Administration, Washington 25, D. C.
DRUGS AND SPICES:
W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.
DAIRY AND EGG PRODUCTS:
Dorothy Scott, Food and Drug Administration, Washington 25, D. C.
NUT PRODUCTS:
Maryvee G. Yakowitz, Food and Drug Administration, Washington 25, D. C.
BAKED PRODUCTS, CEREALS, AND CONFECTIONERY:
J. F. Nicholson, Food and Drug Administration, Washington 25, D. C.
BEVERAGE MATERIALS:
F. A. Hodges, Food and Drug Administration, Washington 25, D. C.
FRUIT PRODUCTS:
W. G. Helsel, Food and Drug Administration, Washington 25, D. C.
VEGETABLE PRODUCTS:
F. R. Smith, Food and Drug Administration, Washington 25, D. C.
SEDIMENT TESTS (MILK AND CREAM):
C. R. Joiner, Food and Drug Administration, St. Louis 1, Mo.
MISCELLANEOUS MATERIALS:
A. H. Tillson, Food and Drug Administration, Washington, D. C.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

*Referee:* John B. Wilson, Food and Drug Administration, Washington 25, D. C.
BETA-IONONE:
John B. Wilson
PEEL OILS IN CITRUS JUICES:
John B. Wilson
ORGANIC SOLVENTS IN FLAVORS:
R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.
EMULSION FLAVORS:
John B. Wilson
MAPLE FLAVOR CONCENTRATES AND IMITATIONS:

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VANILLA EXTRACTS AND IMITATIONS:
I. Ensminger, Food and Drug Administration, Cincinnati 2, Ohio
PROPYLENE GLYCOL:
C. F. Bruening, Food and Drug Administration, Chicago 7, Ill.

FRUITS AND FRUIT PRODUCTS:

*Referee:* R. A. Osborn, Food and Drug Administration, Washington 25, D. C.

FRUIT ACIDS:

L. W. Ferris, Food and Drug Administration, Buffalo 3, N. Y.

FROZEN FRUIT (FRUIT, SUGAR, AND WATER):

H. O. Fallscheer, Food and Drug Administration, Seattle 4, Wash.

FROZEN FRUIT (FILL OF CONTAINER):

W. W. Wallace, Food and Drug Administration, Seattle 4, Wash.

PRESERVATIVES AND ARTIFICIAL SWEETENERS:

*Referee:* Margarethe Oakley, State Department of Health, Baltimore 18, Md.

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MONOCHLOROACETIC ACID:

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THIOUREA:

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SUGARS AND SUGAR PRODUCTS:

*Referee:* C. F. Snyder, National Bureau of Standards, Washington 25, D. C.

DRYING METHODS:

Lester D. Hammond, National Bureau of Standards, Washington 25, D. C.

DENSIMETRIC AND REFRACTOMETRIC METHODS:

C. F. Snyder

HONEY:

Jonathan W. White, Jr., Eastern Regional Research Lab., Philadelphia, Pa.

REDUCING SUGARS:

Emma J. McDonald, National Bureau of Standards, Washington 25, D. C.

CORN SIRUP AND CORN SUGAR:

G. T. Peckham, Jr., Clinton Company, Clinton, Iowa

TRANSMITTANCY OF SUGAR SOLUTIONS:

F. W. Zerban, N. Y. Sugar Trade Lab., 113 Pearl St., New York, N. Y.

MICRO-SUGAR METHODS:

Betty K. Goss, National Bureau of Standards, Washington 25, D. C.

STARCH CONVERSION PRODUCTS:

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WATERS, MINERAL AND SALT:

*Referee:* C. G. Hatmaker, Food and Drug Administration, Washington 25, D. C.

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## PRESIDENT'S ADDRESS*

# PROGRESS IN CONTROL TESTING OF VITAMIN D SUPPLEMENTS FOR POULTRY

By H. A. HALVORSON (Department of Agriculture, Dairy and Food, St. Paul, Minnesota)

In his address before this group last year, President Queen spoke of the many "cherished traditions" belonging to our Association. Surprisingly, during the past few months, when faced with the problem and the necessity of preparing a suitable address for the present occasion, I felt very much impelled to heartily agree with him that the custom for the president to address the annual meetings was not, as he expressed it, one of these "cherished" occasions. My feeling on this matter gradually changed from one of sharp disagreement with our previous president as I listened last fall to his interesting paper, to one of sympthetic understanding now of his reasons for saying this. As was pointed out a year ago, the president is free to select his own subject, and I have accordingly chosen to present a very sketchy historical review of what has taken place since 1930 in the testing of commercial products used for furnishing vitamin D in poultry rations.

Naturally, in the course of twenty years a great many changes and much progress will occur, especially if the subject has both a scientific and economic significance. A tremendously large number of papers on the subject of the chick assay method for vitamin D in its various phases have been published, not only in *This Journal*, but in many others, including *Science*, *Poultry Science*, and the *Journal of Biological Chemistry*. In a short address, therefore, it is possible to refer to only a very few of these papers.

Practically none of the commercial poultry rations marketed in this country prior to 1930 included cod-liver oil or other vitamin D supplement as ingredients, although it was known before that time that cod-liver oil, because of its comparatively high vitamin D content, was effective in the prevention of rickets and helpful in the development of strong and healthy bones. The discovery of the value of cod-liver oil as a curative agent and for the prevention of rickets belongs to no particular person, since its efficacy in this respect was recognized in quite ancient times. In the 1920's several investigators published papers in American and European journals which set the stage, so to speak, for investigations which later led to the use of cod-liver oil and other vitamin D preparations in commercial feeds. Among these investigators, and the titles of the papers which disclose the significance of their contents, are the fol-

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lowing: Dr. Ruth Guy (1923), "The History of Cod-Liver Oil as a Remedy"; C. E. Bills (1927), "The Distribution of Vitamin D with some Notes on its Possible Origin"; Clow & Marlatt (1929), "The Anti-rachitic Factor in Burbot Liver Oil"; Drummond & Hilditch (1930), "The Relative Values of Cod-Liver Oil from Various Sources"; McCollum and colleagues (1922), "A Delicate Biological Test for Calcium Depositing Substances."

Thus it will be seen that in the decade which ended in 1930 it was well known among scientists that cod-liver oil and a number of other fish body and liver oils (notably from the menhaden, tuna, salmon, and sardine), when produced under properly controlled conditions, contained relatively high vitamin D potencies. Other substances were also recognized as good sources of vitamin D, and these included egg yolk, butter, clams, whole milk, and oysters, although these latter were not regarded as always dependable supplements nor were they practical products for use in feedstuffs. At the beginning of the 1930's, vitamin D in high concentrations began to be supplied in the form of artificial concentrates such as the non-saponifiable fractions of cod-liver oil; also in the form of ergosterol, which had been irradiated with ultraviolet rays, and other irradiated food and feed substances. The feed bulletins of the several States in northern latitudes will generally bear out the statement that vitamin D supplements and cod-liver oils were not contained in the lists of ingredients of feedstuffs prior to 1925. After that year and before 1930 a few venturesome manufacturers included cod-liver oil in certain poultry rations on an experimental basis. The experiments conducted by Dr. E. B. Hart and others at Wisconsin and elsewhere, in which animals were exposed to ultraviolet light and sunshine, definitely proved that animals and poultry grown and used for reproduction in northern latitudes needed more artificial "sunlight" in the form of vitamin D, exposure to ultraviolet light, or a diet which had been exposed to ultraviolet rays, than did similar animals grown and produced in southern latitudes.

About the year 1930 and possibly in some years before that time, producers of vitamin supplements employed in the feed industry and even some of the feed manufacturers themselves, sought to convince purchasers that their products were as potent from the vitamin standpoint as their competitors' feeds, or as valuable as experiment station diets which had been publicized in newspapers and farm journals. Coincident with this development was the fact that the poultry industry itself was passing through a revolution. Efforts on a large scale were being made by producers to increase egg production during the late fall and winter months, to hatch and raise baby chicks during these dark months, and to fatten young poultry for fryers for use at a time of year when previously only cold storage poultry had been available for hotel, restaurant, and home use. As a consequence, the poultry industry wanted and demanded for

winter use feed rations which would yield better or as good hatchability and livability as the ordinary rations would produce in the spring and summer months. In this quandary the feed manufacturer turned to the experiment stations and the producers of vitamin supplements, both natural and irradiated. On the whole, when viewed from our present standpoint and considering the dearth of reliable data then available, the results produced by those early supplemented feeds were fairly good. At the time, however, the consequences many times seemed calamitous when poultry raisers often encountered severe disappointments and losses in their flocks.

One of the principal causes of losses of winter-hatched baby chicks, which were fed vitamin fortified diets in the early 1930's, was the great variation in the vitamin D contents of the supplements then available. This was in spite of the fact that as early as 1926 Heuser and Norris (1) called attention to apparent variations in the vitamin D content of a number of commercial cod-liver oils. The variety of the products on the market for supplying vitamin D to rations is illustrated by the early inspection work reports of a typical northern State which include cod-liver oil, concentrated cod-liver oil, sardine oils, proprietary mixed products claimed to have antirachitic properties, cod-liver cake meal, burbot liver oil, cod-liver oil stearines, and irradiated yeast products. It is significant now that over half of the samples tested in the three years ending with 1933 were straight cod-liver oils in which the vitamin D contents were generally lower than the 85 units per gram required by definition.

A further cause for disappointment in commercial feeding results was the lack of uniformity in guarantees on labels and circulars for the antirachitic products recommended. Examples of statements on labels used to describe such materials include the following: "extremely potent in vitamin A and D," "biologically tested in our own laboratories," and "guaranteed to conform to all U.S.P. requirements." One product bore the notation "not U.S.P. but high in vitamin D content." Another was labeled to have "14,000 units vitamin A and 7,000 units vitamin D," without specifying the kind of units or the weights in which they were contained. One brand of concentrated cod-liver oil was uniformly labeled with the statement "$\frac{1}{8}$ per cent level." This was interpreted at that time to mean that the material was eight times the strength in vitamin D of the average or standard cod-liver oil since the latter product was usually then recommended to be fed at the one per cent level. Naturally, most of these products, when tested, failed to meet their guarantees.

Uncertainty regarding the amounts to use and confusion over significance of the guarantees and statements on labels and in circulars resulted largely from lack of agreement or understanding of the meaning of the terms employed. Many units for measuring the antirachitic value by means of a rat feeding technique had been proposed by scientific workers

as well as by research chemists in commercial laboratories. Often the names of the units employed in the label guarantees were lacking and it was not surprising that purchasers were at a loss to know what brands and what amounts to use.

As late as April 1934 there were at least seven different rat units for the measurement of vitamin D which had been proposed by scientific workers and in many cases used by them and various manufacturers. In view of this situation the author, and his associate, L. L. Lachat, prepared the attached table of rat unit equivalents which defined the strength of the seven proposed units in terms of the other six on the basis of the equivalent value of one of each of the following units: International unit, U.S.P. unit, Steenbock unit, American Medical Association unit, American Drug Manufacturers' Association unit, the D unit, and the Oslo unit.

*Rat unit equivalents*

| | INT'L. UNITS | USP UNITS | STEENBOCK UNITS | AM. MED. ASSN. UNITS | AM. DRUG MFRS. ASSN. UNITS | D UNITS | OSLO UNITS |
|---|---|---|---|---|---|---|---|
| One International unit equals | — | 1.00 | 0.37 | 0.37 | 3.25–3.70 | 0.028 | 1.67 |
| One U.S.P. unit equals | 1.00 | — | 0.37 | 0.37 | 3.25–3.70 | 0.028 | 1.67 |
| One Steenbock unit equals | 2.70 | 2.70 | — | 1.00 | 10.00 | 0.075 | 4.50 |
| One Am. Med. Assn. unit equals | 2.70 | 2.70 | 1.00 | — | 10.00 | 0.075 | 4.50 |
| One Am. Drug Mfrs. Assn. unit equals | 0.27 | 0.27 | 0.10 | 0.10 | — | 0.008 | 0.45 |
| One D unit equals | 36.00 | 36.00 | 13.33 | 13.33 | 133.30 | — | 60.00 |
| One Oslo unit equals | 0.60 | 0.60 | 0.22 | 0.22 | 2.22 | 0.017 | — |

Since prior to 1933 neither the A.O.A.C. nor the Association of American Feed Control Officials had published their own standards and definitions for most of the terms relating to vitamin D supplements for animal use, Merck's Index and the New and Non-Official Remedies of the American Medical Association were common references. These sources defined: Ergosterol, as a sterol isolated from ergot or from yeast; Viosterol, as irradiated ergosterol (activated ergosterol); Viosterol in Oil, as irradiated ergosterol in oil; one gram of Viosterol in Oil 250 D, as containing 3,333 rat units of vitamin D (the strength was 250 times that of a potent cod-liver oil used as a standard). These sources also defined one rat unit to be "the amount of vitamin D which when uniformly distributed in the standard vitamin D deficient ration would produce a narrow and continuous line of calcium deposits in the metaphyses of the distal ends of the radii and ulnae of standard rachitic rats." A "Cod-liver Oil

of Definite Potency" was also defined briefly to contain one rat unit of vitamin D in 75 milligrams.

A quotation from one State publication reported the results of assays in 1934 as follows, and thus vividly portrayed the state of disorder on this whole subject at the time and the steps being undertaken to remedy it: "The policy of the Association of American Feed Control Officials as expressed in the resolution adopted at its annual meeting in November, 1933, is expected to eventually bring order out of the confusion which has existed with reference to the labeling of products sold as vitamin D carriers. This resolution provides: ' . . . that any material sold primarily as a vitamin D supplement for poultry shall be labeled with a minimum guarantee of antirachitic potency, specifying the minimum percentage level at which normal calcification is produced, according to the tentative method of analysis as adopted by the Association of Official Agricultural Chemists.' The action of that organization and the steps taken by several States . . . in requiring the filing of registrations and the labeling of these products with minimum guarantees of potency, placed them in a class with other feeding materials except for the fact that the guarantees were expressed in other terms than in protein, fat, and crude fiber."

In addition, these definite guarantees for antirachitic value, made on the labels and in registrations filed under oath, greatly lessened the work required in the assay of these materials. Thereafter, tests needed to be made only at the percentages guaranteed by the distributor to give full protection against rickets. The resulting reduction in cost, equipment needed, and work involved in the assay of each sample permitted the testing of a larger number of products each year.

From the foregoing it will be noted that definitions, standards, testing methods and most available information regarding the potency of cod-liver oil and other sources of vitamin D were at first based on the rat feeding technique. Although Hart, Halpin and Steenbock (2) had shown, as early as 1922, the chick's large demand for vitamin D in the form of cod-liver oil for the prevention of rickets, the first person to suggest the use of a chick feeding technique as a measure of value from the feed control standpoint was W. B. Griem of Wisconsin. In November 1931 Greim (3) proposed to this Association the adoption of a chick feeding method for the assay of cod-liver oil and allied materials for vitamin D potency. For a year or two prior to that date he also had experimented with the procedure and the composition of a suitable basal diet, in cooperation with Dr. Hart. In this early period it was not definitely known that there were several forms of vitamin D and that the antirachitic factor in certain irradiated products and in some fish oils was not nearly as effective in preventing rickets in chickens as in rats and other four-footed animals. Also at that time the method of testing specified the percentage of the tested material to be used in the basal ration at three levels, *i.e.* equal

to, above, and below the recommended level of the manufacturer. This was all because we had no definite unit which could be applied to chickens. Subsequent to the above period Bills (4) and associates in 1934 published a paper suggesting that there were at least two forms of vitamin D and stated that the vitamin D in bluefin tuna-liver oil is a different substance or a different mixture of substances than the vitamin D in cod-liver oil. For chicken use they found that one rat unit of the antirachitic factor in bluefin tuna-liver oil was only about 15 per cent as effective as that in cod-liver oil. About the same time Waddell and Rohdenburg (5) showed that irradiated cholesterol is responsible for greater antirachitic potency than is irradiated ergosterol for preventing rickets in chickens. In other words, irradiated cholesterol in contrast with irradiated ergosterol is as effective for poultry (rat unit for rat unit) as the vitamin D in cod-liver oil.

As early as 1930 the Association of American Feed Control Officials adopted a definition for cod-liver oil which reads as follows: "Cod-liver oil is the product obtained by extraction of part of the oil from cod livers. It shall be labeled Vitamine D Sub-Standard Cod Liver Oil if its vitamine potency, when measured by the Association of Official Agricultural Chemists tests, fails to produce the minimum calcification as accepted." While this definition helped to bring some order out of derangement it was not until this Association adopted the definition for the A.O.A.C. chick unit in 1936 (6) that it was possible to legally require definite guarantees in terms of vitamin D units on labels for products sold primarily to supply this vitamin in commercial poultry rations. Hence the discovery that there were different forms of vitamin D in a variety of products both natural and irradiated; the adoption of the definition and standard for a chick unit; and the acceptance of a definite method of assay by the A.O.A.C., filled out the picture and enabled all feed control officials everywhere to adopt uniform regulations, definitions, and labeling requirements for these materials.

The fact that the U. S. Pharmacopoeia Vitamin Advisory Board had made available a "reference cod-liver oil" of a definite and accepted potency, and the fact that it was possible to assay this oil in 1934 for the first time by the recently adopted A.O.A.C. chick method (7) helped immeasurably to place the testing program for poultry vitamin D products on a sound basis. In the years following 1930 with the various developments just cited, laboratories for the assays of vitamin D products were established or greatly expanded in a number of the States and by the Federal Government. A survey made early in 1950 by Griem of Wisconsin for the Association of American Feed Control Officials showed that vitamin D assay work was being performed in fifteen States and the Dominion of Canada. In four of these sixteen laboratories the report indicates that the volume of testing work done requires two full time employees. The other

twelve States devoted less time to the program. Eight laboratories reported making vitamin $D_3$ chick assays on samples taken from an average of 62 shipments per year. However, a more recent development indicates some slowing down and even discontinuance of the work of assaying for vitamin $D_3$, possibly for the reason that the cost per unit of this vitamin is much less now than formerly because of the availability of irradiated cholesterol products. Naturally, as this vitamin becomes cheaper there is less incentive for adulteration.

In a paper before this Association in October 1949, Dr. E. M. Nelson (8) discusses fully the new standard for vitamin D and includes "The World Health Organization has adopted pure vitamin $D_3$ as the International standard for vitamin D in place of the irradiated ergosterol preparation that has been in effect since 1931. For use in this country the U.S.P. Vitamin Advisory Board has recommended the adoption of a similar vitamin $D_3$ standard that will replace the U.S.P. reference cod-liver oil . . . . The results of these studies and also chemical and physical studies show that vitamin $D_3$ is suitable as a standard for vitamin D and that it has a potency very close to 40 million units per gram. At a meeting of a Committee on Fat-Soluble Vitamins, . . . it was recommended . . . that vitamin $D_3$ be adopted as the International Standard for Vitamin D, and the unit be defined as the vitamin D activity of .025 micrograms of vitamin $D_3$. . . . The adoption of vitamin $D_3$ as a standard will put the chick unit on a permanent basis because it will be based on a definite weight of a pure and readily reproducible material . . . ." The extremely high potency of this standard may be better comprehended when it is explained that only a single gram of it properly dispersed in forty tons of chick starter mash would provide a liberal margin of safety against the development of rickets. Thus we see that in the twenty-year period ending in 1950 the necessity and desire for doing something constructive about the trying situation existing around 1930 has, through the cooperation of the various organizations and individuals concerned, resulted in the development and adoption of an almost universally accepted standard of measurement of value for a very necessary and important new product used in agriculture.

For summarizing and concluding the inadequate review here presented I feel greatly indebted to Dr. James C. Fritz of the Borden Company for suggesting the following very pertinent statements and several additional significant points: **(a)** Recognition of the importance of vitamin D made possible our present year-round poultry production; **(b)** Species differences in the ability to utilize various sterols with vitamin D activity made it necessary that an assay method based upon response of birds be developed; **(c)** The A.O.A.C. chick assay method does give reliable results, but the limitations of biological variation must be considered when interpreting data; **(d)** Collaborative studies have been concerned chiefly with

improvement of the basal diet and seem to have ignored the probably greater variable of the chicks themselves. Continued investigation along the latter line is likely to offer the best promise of increasing the accuracy of the chick assay method; **(e)** Various criteria of the chick assay, such as tibiae ash, toe ash, and TMT distance, all give about equally accurate evaluations; **(f)** Chemical methods may be practical for very high potency materials, but offer little hope for use on low potency poultry feed supplements; **(g)** Recognition of the fact that vitamin D is subject to oxidative destruction made possible the development of stable, dry vitamin D carriers. The chick assay method made these studies possible; **(h)** Irradiation of animal sterols for the production of vitamin $D_3$ assures an ample supply of vitamin D. The A.O.A.C. chick assay was essential to this development; **(i)** Recent economies in the production of vitamin $D_3$ have greatly reduced its cost, resulting in less criticism of the method because manufacturers can now better afford to use adequate margins of safety; **(j)** Vitamin D requirements of most species are now well established and relationships between calcium, phosphorus, and vitamin D have received detailed study.

Therefore little excuse now can be offered for rickets in farm animals and poultry.

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# THE A.O.A.C. AS AN AID TO FOOD AND DRUG REGULATION*

By CHARLES W. CRAWFORD, Commissioner, Food and Drug Administration, Federal Security Agency, Washington, D. C.

I am glad to talk to you today about the fundamental and indispensable part you have played and are continuing to play in protecting the health and welfare of the public from the hazards of harmful and fraudulent foods, drugs and cosmetics.

It was the development of methods of analysis which revealed conclusively the nature of the frauds practiced in the days before the Food and Drugs Act of 1906, and which dramatized the need for an adequate federal statute. Without the findings of his laboratory, Dr. Harvey W. Wiley, who had such an important part in the development of this Association, could hardly have succeeded in his crusade for public protection. It was only natural that the plan of regulation which eventually developed should have been based on scientific determination of facts obtained by analysis of authentic materials.

Other approaches could have been tried. Inspection at the source has increasing usefulness in regulatory work. It is always helpful. If adequate analytical methods are not available, it is indispensable. But its limitations are obvious. It would be impractical as well as undesirable for government to monitor by inspection alone production of the more than $55 billion worth of foods, drugs and cosmetics which annually flow from fields and factories through all the channels and rivulets of commerce. In the absence of complete inspection evidence analytical methods are essential. Even if complete inspection data are available, confirmation by analysis may be required to insure successful court action. Today, as a generation ago, the primary tools of regulation are provided by the analytical laboratory.

In a very real sense, the chemical analyst was the first to apply scientific methods of crime detection, now widely used by other law enforcement agencies. In the Wiley era the analyst was a popular hero of Sunday Supplement science. He was in the limelight as the "laboratory sleuth" who tracked down the malefactor with test tubes and brought him to justice.

I recall very vividly the dramatic impact of the exhibits prepared by Dr. Wiley and his associates that I saw at the St. Louis World's Fair in 1904 when I was a high school student. Bits of wool brightly dyed with coal-tar colors extracted from foods made one wonder what happened to one's insides when the foods were eaten. The horrors of foods embalmed with preservatives were strikingly presented. Economic adulterations of

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 1–3, 1951.

many kinds, such as the substitution of pepper shells for pepper, were shown. These exhibits inspired my first interest in food and drug work.

The genesis of the A.O.A.C., as an organization, is traced to the need for methods of analysis to enforce the first regulatory laws designed to protect the buyer through the medium of a truthful label. Those were the laws which the several States enacted to require the labeling of fertilizers as to their true value in terms of the plant foods they contained. These laws were followed by the passage of others to require the labeling of animal feeds to show their nutritive values. This reflected the curious fact that widespread interest in animal nutrition preceded by many years a comparable breadth of interest in human nutrition. During the long legislative battle that preceded the passage of the Food and Drugs Act of 1906, Dr. Wiley remarked ironically that if you want to be well nutrified, be a hog.

Incidentally, these laws were among the first to attack the doctrine of *caveat emptor*. The passage and enforcement of the subsequent and numerous federal and state laws controlling foods, drugs, cosmetics, economic poisons and other commodities vastly expanded the principles of those early fertilizer and feed laws to include control of many abuses, some then apparent and others not yet foreseen. The development of the law has completed the relegation to the scrap heap of unsound jurisprudence, of that legal refuge of the seller who takes unfair advantage of the buyer. Courts no longer give credence to arguments postulated on the right of the producer or dealer to ignore truth, honesty, and fair dealing. In fact, they have repeatedly stressed the rights of the consumer to honest merchandise, honestly labeled, and have interpreted the laws in the consumer's interest rather than in the dealer's. The United States Supreme Court has observed that, in the circumstances of modern industrialism, consumers are largely beyond self-protection.

The activities of this Association in developing the tools for enforcement have contributed in large measure to the decline of the doctrine of *caveat emptor*. Your methods have met the final test of litigation. Over the years, the cases which failed for want of adequate analysis where A.O.A.C. methods were used, have been extremely few. One need only to compare the present 7th Edition of *Methods of Analysis* with the earlier ones, or with the old Bulletin 107, to appreciate the progress made in the development of methods to meet demands continually created by new problems. While the problems appear new, the fundamentals remain unchanged. One trait of human nature is to seek advantage. It is not unexpected that natural motives activate those sellers who are not inhibited by scruples, to extol their products by citing results by that method that will put them in the most favorable light.

As a present example of such a situation, from the scores of examples we could choose, let us consider the question of sodium in foods. It has

been found that lowered sodium intake may alleviate high blood pressure. Manufacturers promptly sought to meet the demand thus created for foods low in sodium. Many so-called salt-free foods are being offered, although the term "salt-free" is not well chosen for foods free only of added salt. Inasmuch as it is the sodium intake which should be restricted some manufacturers offer foods in which the normally occurring sodium has been reduced. As the recommended consumption of sodium per day is quite low it becomes necessary that the sodium content of the food be stated on the label to enable the consumer to stay within established bounds. The statement must be true to fact. It is not sufficient to give on the label a figure based on average compositions reported in the literature. Careful analytical control is required to determine what the statement should be. Faced with a choice of methods some manufacturers may choose that which gives the lowest result, or expediency may lead to the use of a rapid method that gives uncertain results.

The regulatory official, in fulfilment of his responsibilities for public protection, must regard accuracy as of paramount importance in checking label statements of sodium content. The method employed must be one of demonstrated reproducibility. It should give concordant results in the hands of qualified analysts so that the manufacturer through its use is able to determine the true composition and thus avoid violation of the law through inaccurate label declaration. The development of such a method also involves considerations of rapidity, ease of manipulation, use of conventional equipment, and like factors.

To assure promptness in the establishment of appropriate methods, the correlation of the efforts of regulatory and industrial scientists is advantageous. But it has long been recognized that final decision as to whether a method merits recognition and adoption should rest upon the regulatory official, in view of his responsibilities under the law.

This Association has established itself, throughout its history of 67 years, as the 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 safety and wholesomeness and integrity of the Nation's supplies of such vital commodities as its foods and drugs.

### 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.

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**Third Day**

## WEDNESDAY—MORNING SESSION

### REPORT OF THE EDITORIAL BOARD

HENRY A. LEPPER, *Chairman*

At our last meeting, the progress being made on the publication of the seventh edition of *Methods of Analysis*, was reported. We then expressed our disappointment at not having at least one finished copy to demonstrate. It was, however, not more than one month after our meeting when the book was completed and the filling of orders begun. Thus the publication of the seventh edition was accomplished in 1950, the year in which the revision was due, an accomplishment unprecedented with the previous six editions. From the present data before us it appears that this edition may also be freer of errata than any previous one. A list of errata will be published in the forthcoming February number of our *Journal*. Reviews of the book have appeared in many domestic and numerous foreign journals, scientific, technological, and industrial, in which it is regarded as an outstanding contribution to fields to which it is dedicated. Its reception by the profession and others has been most gratifying attested to by the sale of six thousand copies this first year.

Mr. Paul Clifford, the Editor of the *Journal*, will give the report of the Editorial Committee; and Dr. H. J. Fisher will give the report of the Committee on Revision of *Methods of Analysis*.

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### REPORT OF THE EDITORIAL COMMITTEE OF THE *JOURNAL*

PAUL A. CLIFFORD, *Editor and Chairman*

Volume 34 of the *Journal* will run to about 860 pages, with 44 contributed papers and notes, and 18 book reviews. In order to meet our quarterly publication dates, it is now necessary to submit all copy to the printer two months in advance; accordingly, a number of papers presented at this meeting must await publication some time next year.

The number of *Journal* subscriptions remains about the same; it was

1862 in 1949, 1857 in 1950, and 1851 in September of this year. We are sure that this condition does not indicate a "saturated market" and we would like to repeat the admonition of our former Editor, W. B. White, that each subscriber act as a "personal salesman" and "point out the value of the *Journal* to every prospect." The broadening activities of the Association should interest workers in a great number of allied fields. *Chemical Abstracts* lists 33 major chemical topics. The fact that more than half of these are proper A.O.A.C. subject matter illustrates the variety and scope of our work.

Publication of a scientific journal is expensive. It cost us $2979.00 to print the last August number (273 pages) and printing costs have recently risen another 12 per cent. The *Journal* continues to operate in the red.

There are two obvious ways to remedy this situation; we have already mentioned the desirability of obtaining new subscribers. Three hundred or even 200 new subscriptions would help greatly in placing the *Journal* on a paying basis. Another way would be to limit its size.

In this respect, referees and associate referees could help greatly by abbreviating their reports. Many reports are well-written but others are poorly organized and "full of words." It has been the custom in the past to allow the referees nearly *carte blanche* in the presentation of their reports; in many cases, more careful editorial scrutiny would be desirable. They should be as brief as is consistent with clarity. Some referees fill several pages with the comments of collaborators. The cooperation of collaborators should, by all means, be acknowledged and their comments, when pertinent, should be quoted. However, these are often presented in an informal, even garrulous, style and much space could be saved if the referee, rather than giving them verbatim, would present them in abstract. Tables (such as those giving collaborative data) should be clearly set out in a minimum number of vertical and cross columns and should be given a short but descriptive caption. Graphs and charts should be carefully drawn, preferably with India ink on tracing cloth, and lettering should be large enough to be readily legible when the graph is reproduced at half-page size (approximately $4 \times 4''$). Carefully drawn and lettered charts add to the appearance of a paper and perhaps the reader, whether rightly or no, takes them also as evidence of careful analytical work. On the other hand, crudely drawn and carelessly hand-lettered charts on ordinary graph paper are sure to give a reverse impression. (Besides, they do not reproduce well.)

The outline of an associate referee's report should follow that of scientific papers in general. A suggested scheme is: (1) *A brief statement of the problem and of progress made to date.* (2) *An outline of the method used.* If results obtained do not warrant a recommendation for adoption of the method, this outline should be made very brief. However, the method

should be detailed (reagents, apparatus, procedure, etc.) if accompanied by a recommendation for adoption. (3) *Presentation of collaborative work.* This involves a description of the collaborative samples and a simple tabulation of results. Statistical analysis of these can be briefly summarized. Collaborators' names and affiliation can be given in a footnote to a table or page. (4) *A short appraisal of the results obtained.* Here collaborators' comments may be summarized, *e.g.*,—"most collaborators expressed approval of the method" or "collaborator B had difficulty with the purification of reagent (i)"—etc. (5) *The report should close* with a recommendation either for adoption of the method or for further work. In the latter case it is well to indicate plans, such as certain modifications of the method or trial of a new method.

Chemists have complained that simple titles such as "Report on Copper," "Report on Decomposition," etc., often do not adequately describe the work done and that important cross-references may be overlooked when the report is abstracted. In many cases it would be best to give a supplementary descriptive title such as, "Report on Uric Acid. Application of Paper Chromatography" or, "Report on Mercury. Determination of Trace Amounts in Foods by Means of Dithizone."

Only about 36 per cent of the contents of Volume 34 will consist of contributed papers; the bulk is still taken up with referee reports. Let us try to increase their worth, both scientific and literary. We deal with 40 main topics and have 232 referees and associates. All assignments are based upon a current need for new or improved methods and should challenge the efforts of the most competent investigators. Referees should remember that their reports are published, under their names, as contributions to the permanent scientific literature. And we would like to dissuade them from any view that their reports lack something of the status of contributed papers. Is this false impression due to the fact that some referees slight their work and that their reports, consequently, are sometimes lacking in substance?

We hope that general referees and laboratory supervisors will continue to assist their associates and subordinates, especially those newly appointed, not only with their assigned work but also in the preparation of their reports. We again extend our thanks to all referees, contributors, and reviewers whose efforts are responsible for a steady gain in the prestige of the *Journal.*

Approved.

## REPORT OF COMMITTEE ON REVISION OF *METHODS OF ANALYSIS*

The committee's work was completed with the printing of the seventh edition of *Methods of Analysis*. Two years from now a new committee should be appointed to take over preparation of the eighth edition. In the meantime, I move that the present committee, since it has no function to perform, be discharged.

HARRY J. FISHER, *Chairman*

Approved.

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

A Committee on the Approval of Culture Media has been created in the APHA. Since the same extract and/or digest type media will probably be approved for plating many substances, including foods other than dairy products, and since a synthetic medium, similar to the one reported on last year, will probably be recognized as a colony productivity reference base for determining the relative productivity of nonsynthetic media, it was deemed advisable to form this new committee.

Among the first objectives will be a determination as to whether essentially the same relationship, as observed in 1950 between the productivity of the synthetic medium and the official tryptone, glucose, beef extract, skim milk medium, can be reproduced using another group of milk samples. Studies will include attempts to improve and simplify the synthetic medium. After selection of suitable skim milk-free media to replace the present milk-containing media, steps will then be taken to approve for official use successive factory batches of these media after their productivities have been determined to conform with a recognized standard. This new activity may require several years before the administrative responsibilities will be clarified.

Since 1945 several new methylene blue stain formulas have been proposed as substitutes for the time-honored formula for the direct microscopic method of determining the bacterial content in milk and other dairy products. Collaborative studies are now in progress to determine the worthiness of each of six staining mixtures and their different applications to milk and cream films dried on micro glass slides.

Your Referee, who is also designated by the APHA for sediment in milk, has recognized the need for a finer mixture than that described in **35.9** for the preparation of standard discs to compare with the amount of sediment on test discs obtained using retail milk samples. Using this mixture, an attempt has been made to draw up minimum specifications

for a suitable cotton disc in terms of the amount of a prescribed sediment charge which it will retain. If the sediment is too fine, too much of it will pass through the disc, and if the disc is too thick, it will unduly retard the flow of milk. These are practical considerations which govern the selection of a disc.

Collaborative work on residual phosphatase in milk and cream, as originally planned in 1951, has been delayed partly because of unexpected assignments of our jointly named Referee and partly because of pending improvements on a one-hour field method for phosphatase determination. It is expected that the needed comparisons will be completed within a few months.

Likewise, one or more modifications of the Babcock procedure for the determination of fat in homogenized milk will be studied. One well known State Association of Milk Sanitarians and several scattered public health laboratories have requested this study, hoping that a suitable modification will soon be recognized.

A. H. ROBERTSON, *Chairman*
WM. HORWITZ
J. O. CLARKE

Approved.

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## REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

WM. F. REINDOLLAR, *Chairman*

This year the A.O.A.C. Conference, scheduled for the first three days in October, is meeting earlier than at any date in recent years. It is gratifying to note that in spite of this consequent reduction in time available for collaborative studies, the majority of referees and associate referees have submitted their reports sufficiently early for proper consideration by the several subcommittees. The importance of such action cannot be overemphasized; it is essential, not only to those who have the responsibility of reviewing the work and appraising the recommendations, but to the smooth functioning of the entire program.

One task that becomes more difficult with each succeeding year is to secure competent analysts who are willing to participate in the rapidly expanding work of the Association. The A.O.A.C. exists solely for the purpose of creating or evaluating methods to be employed in those fields which it covers. To discharge this responsibility adequately referees, associate referees, and particularly, collaborators must be found. The most logical place to obtain them is from the ranks of chemists who profit by the use of the *Book of Methods*. These individuals cannot be drafted; it is encumbent upon them to volunteer.

As stated repeatedly in past reports the successful functioning of this Committee is the result of the labors of many workers and grateful acknowledgment to them is herewith given.

Approved.

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## 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 the following subjects be discontinued:

(a) Mineral mixed feeds (calcium and iodine)
(b) Lactose in mixed feeds
(c) Protein evaluation in fish and animal products.

(2) That study of the following subjects be initiated:

(a) Crude protein in feeding stuffs
(b) Ash in feeding stuffs
(c) Milk by-products in mixed feeds.

(3) That the work on the following be continued:

(a) Fat in fish meal
(b) Crude fat or ether extract
(c) Microscopic examination
(d) Mineral constituents in mixed feeds
(e) Tankage (hide, hoof, horn, and hair content)
(f) Drugs in feeds.

(4) That the method for sulfaguanidine adopted as first action last year, be made official.

(5) That the study of methods for nitrophenide (m,m'-dinitrodiphenyldisulfide) and enheptin® (2-amino-5-nitrothiazole) be continued, as recommended by the Associate Referee.

(6) That the method for the determination of cobalt in mineral feeds, as outlined by the Associate Referee, be made first action.

### FERTILIZERS

It is recommended—

(1) That the method for water-soluble magnesium in other materials, including mixed fertilizers, **2.57(b)** now first action, be made official.

(2) That the study of magnesium and manganese in fertilizers be continued.

* These recommendations submitted by Subcommittee A were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1950.

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

(4) That work on methods for phosphoric acid in fertilizers be continued, as recommended by the Associate Referee.

(5) That the study of the Shuey method for the determination of nitrogen in high nitrate-high chloride mixtures be continued.

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

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

(8) That the studies of the other Associate Referees on fertilizer analyses be continued.

ECONOMIC POISONS

It is recommended—

(1) That the Toops and Riddick and the Arceneaux methods for the determination of the gamma isomer of benzene hexachloride, and the Fairing and Phillips and the Schecter and Hornstein colorimetric methods for total benzene hexachloride, be studied.

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

(3) That the Davidow and Harris methods for determination of chlordane be studied collaboratively.

(4) That the Elmore method, adopted first action in 1950, be further studied to investigate its applicability for determination of thiocyanate nitrogen in the ester type thiocyanates.

(5) That the ultra-violet absorbance of piperonyl butoxide and its interferences be further studied.

(6) That the partition chromatographic separation of piperonyl butoxide and its determination by the Jones colorimetric method be studied collaboratively.

(7) That collaborative work on determination of warfarin in concentrates be continued.

(8) That work be continued on the development of a procedure suitable for determining warfarin in low percentage warfarin baits.

(9) That methods be studied for allethrin, aldrin, dieldrin, and for the determination of physical properties of economic posions, especially particle size and dispersibility in aqueous and dry formulations.

(10) That the method for determination of potassium cyanate in herbicides be revised as suggested by the Associate Referee and adopted, first action.

(11) That the alkalimetric titration method (revised) for determination of esters of 2,4-dichlorophenoxyacetic acid be subjected to further study.

(12) That the method for determination of total chlorine in liquid herbicides containing 2,4-D, 2,4,5-T, or mixtures of both in presence of

oils and emulsifiers, by a modified Parr bomb procedure, be subjected to further study.

(13) That the Olsen procedure be studied in connection with the methods for 2,4-dichlorophenoxyacetic acid and related compounds

(14) That the changes in the methods for pyrethrins, **5.111, 5.112,** and **5.114,** as recommended by the Associate Referee, be adopted, first action.

(15) That the methods for technical parathion and dust formulations as described by the Associate Referee be adopted, first action.

(16) That the end-point technique of these methods be further investigated.

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

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

### DISINFECTANTS

It is recommended—

(1) That studies on a use-dilution method for testing disinfectants, on the phenol coefficient method, and on the fungicidal test, be continued.

(2) That the study of media for disinfecting testing be continued.

### PLANTS

It is recommended—

(1) That the nitroso-R-salt method for cobalt in plants be further investigated collaboratively, but that work with the nitrosocresol method be discontinued.

(2) That collaborative work on the carbamate method for copper in plants be continued.

(3) That a collaborative study of the ion-exchange resin technique for clarifying plant extracts, for the determination of sugar, be undertaken.

(4) That the study of methods for the determination of zinc in plant materials be continued, and that the modified dithizone procedure be submitted to collaborative study.

(5) That the studies of methods for the following be continued:

(a) Starch
(b) Sodium
(c) Carotene
(d) Iodine and boron.

(6) That study of sampling plant materials for analysis be continued.

### 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 further studies on the *p*H of soils at a moisture content approximating the field capacity be carried out.

(5) That the neutral calcium acetate procedure for the replacement of the exchangeable hydrogen of soils and determination thereof be adopted, first action.

(6) That further studies be made of methods for the determination of the limestone requirement to raise a soil's reaction to a desired practical *p*H value.

(7) 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)).

(8) 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.

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

(10) That the double distillation method for fluorine, *This Journal*, **34,** 58, (1951), as reworded by the Associate Referee, be made official and the work be continued.

### STANDARD SOLUTIONS

It is recommended that work on this subject be continued.

### VITAMINS

It is recommended—

(1) That the method for vitamin A in mixed feeds, *This Journal*, **34,** 97, (1951), be revised as suggested by the Associate Referee, and remain first action subject to further collaborative studies.

(2) That method I for carotene in hays and dried plants, **40.7,** be changed as recommended by the Associate Referee and be adopted as official; and that studies on carotene analysis be continued.

(3) That the study of vitamin D in poultry feed supplements be continued.

(4) That in the chemical method for determination of nicotinic acid, adopted first action last year, *This Journal*, **34,** 99, (1951), the strength of $H_2SO_4$ used for extraction of nicotine acid and conversion of nicotinamide to nicotinic acid be increased from 0.25 *N* to 1.0 *N* and be adopted, first action, and the study be continued.

(5) That a collaborative study be made on the application of the new colorimetric method for vitamin $B_6$ to pharmaceutical products.

(6) That the microbiological method for the assay of added pantothenic acid described by the Associate Referee be adopted, first action.

(7) That collaborative study of the method be continued during the coming year.

(8) That work on the microbiological assay for vitamin $B_{12}$ be continued.

(9) That further consideration be given to sample treatment techniques in this method, and to possible intra-laboratory variations, and that the test materials continue to be crude materials of a wide potency range.

(10) That studies be continued on vitamin A in oleomargarine.

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## REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

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

### RADIOACTIVITY

The committee recommends that the topic be continued.

### SPECTROGRAPHIC METHODS

The committee recommends that the topic be continued.

### VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended that the following topics be continued:

Aminopyrine, ephedrine, and phenobarbital
Quinine and strychnine
Rutin in tablets.

### SYNTHETIC DRUGS

It is recommended—

(1) That the method for propylthiouracil, which includes filtration of the solution prior to spectrophotometric analysis, be adopted, first action; that the topic be closed.

(2) That the following topics be continued, in accordance with the recommendations of the Referee:

Methylene blue
Sulfanilamide derivatives
Propadrine hydrochloride
Spectrophotometric methods
Synthetic estrogens
Di- and triphenhydramine hydrochloride.

* These recommendations submitted by Subcommittee B were approved by the Association. Unless otherwise given all references are to *Methods of Analysis*, A.O.A.C., 1950.

(3) That an associate refereeship be established to study amphetamine and dexedrine sulfates.

### MISCELLANEOUS DRUGS

It is recommended—

(1) That the associate refereeship on alkali metals be continued and reassigned, in accordance with the recommendations of the Referee.

(2) That the topic on methyl alcohol be dropped from miscellaneous drugs, since it is also being studied in relation to beverages.

(3) The microchemical tests for Diphenhydramine Hydrochloride (Benadryl Hydrochloride®), Pyranisamine Maleate, Tripelennamine Hydrochloride (Pyribenzamine Hydrochloride®) as described by the Associate Referee, were adopted, first action.

(4) That the following topics be continued:

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

### COSMETICS

It is recommended—

(1) That the methods for the determination of methenamine and phenolsulfonates be adopted, first action, as recommended by the Associate Referee on deodorants and anti-perspirants, and the subject be continued.

(2) That the qualitative and quantitative procedures for examination of thioglycolate solutions and neutralizers be adopted, first action, as recommended by the Associate Referee on cold permanent wave preparations and the subject be continued.

(3) That the following subjects be continued:

Cosmetic creams
Mascara, eyebrow pencils, and eye shadow
Sun tan preparations
Hair dyes and rinses.

(4) That the topic on cosmetic skin lotions be discontinued.

### COAL-TAR COLORS

It is recommended—

(1) That the following methods be adopted, first action, in accordance with the recommendations of the Associate Referees and the Referee; and the topics be continued:

Subsidiary dyes in FD&C Colors—Lower sulfonated dyes in FD&C Yellow No. 5
Sulfonated amine intermediates in coal-tar colors—Lake Red C Amine in D&C Red Nos. 8 and 9.

(2) That the following topics be continued:

Paper chromatography of 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
Subsidiary dyes in D&D colors (4-toluene-azo-2-naphthol in D&C Red No. 35)
Lakes and pigments
Spectrophotometric testing of coal-tar colors
Determination of arsenic and antimony in coal-tar colors
Boiling range of amines derived from coal-tar colors
Determination of heavy metals in coal-tar colors
Sulfonated phenolic intermediates in coal-tar colors
Intermediates in triphenylmethane dyes.

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## REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

PAUL A. CLIFFORD (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Chairman*; A. H. ROBERTSON; and S. ALFEND

*General Recommendation*: The Committee repeats its recommendation 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 the rapid method described by the Associate Referee for the determination of residual catalase activity in frozen vegetables be subjected to further collaborative study.

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

### COFFEE AND TEA

It is recommended—

(1) That methods for chlorogenic acid in coffee be developed.

(2) That more modern methods for the determination of moisture in coffee and tea be developed.

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* These recommendations submitted by Subcommittee C were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.*, 1950.

### DAIRY PRODUCTS

It is recommended—

(1) (a) That the hand-stirring method described by the Associate Referee for the sampling of commercial containers of cottage cheeses be adopted as a procedure and (b) that in the preparation of laboratory samples the possibility of moisture loss due to the use of high speed mixers be further investigated.

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

(3) That further work be done on methods for the determination of the acidity of colored frozen desserts.

(4) That the method for fat in frozen desserts, **15.153,** be modified to provide for direct addition of the sample to the Mojonnier tube, as described in the Associate Referee's report.

(5) That the directions for the preparation of butter samples be modified as follows and be adopted as official: (a) that the shaking procedure, **15.105,** be amended as suggested by the Associate Referee; (b) that paragraphs **15.106–15.107** be deleted; that **15.104(c),** sample containers, be changed as recommended by the Associate Referee.

That further collaborative work be done with the mechanical shaking method.

(6) That further work be done with the Babcock method as applied to the determination of fat in homogenized milk.

(7) That the following methods be made official: casein in fluid milk, Method II, **15.19–15.20** and casein in malted milk and chocolate malted milk, **15.95**; fat in malted milk, **15.98**; that the official method for residual phosphatase, **15.47–15.49,** be made official for butter, **15.122,** for cheese, **15.144–15.147,** and for ice cream, **15.157.**

(8) That the question of proper interpretation of cryoscopic data, **15.30,** be further considered.

### EGGS AND EGG PRODUCTS

It is recommended—

(1) That further work be done on the quantitative determination of glycerol in mixtures of eggs and sugars.

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

### DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES)

It is recommended—

(1) That the study of galacturonic acid as a measure of decomposition in apples, strawberries, spinach, etc. be continued; that the work be correlated, and expanded, if possible, to include collaborative study.

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

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

(4) 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.

(5) That study of chemical indices of decomposition in shellfish be continued.

(6) That studies of uric acid as an index of insect filth in nuts, cereals, and fruit products be continued.

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

(8) That the first action method for succinic acid, **16.34–16.39,** be adopted as official.

### GELATIN DESSERT PREPARATIONS AND MIXES

It is recommended—

(1) That studies of the composition of gelatin, gelatin dessert powders, and starch dessert powders be continued.

### FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the method described by the Associate Referee for determining total solids in fish and other marine products except oysters be subjected to collaborative study.

(2) That work on the method for determination of total solids in oysters described by the Associate Referee be continued, with investigation of possible loss of moisture in the high speed mixer.

### 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 method for mercury proposed by the Associate Referee be adopted as first action.

(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 and parathion be continued.

(5) That the qualitative and quantitative methods for sodium fluoroacetate (1080) described by the Associate Referee be studied collaboratively.

(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 studies be made of methods for the determination of benzene hexachloride in food.

(8) That studies be made of the determination of fluorine in plant materials of high silica content.

### GUMS IN FOOD

It is recommended—

(1) That the method for the detection of gums in catsup and related products be submitted to collaborative study.

(2) That the method for the detection of gums in salad dressing, as described by the Associate Referee, be adopted, first action.

(3) That work be done on the detection of algin by the Associate Referee on gums in cheese.

(4) That work be continued on the detection of gums in cacao products.

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

(6) That work be continued on 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 the proposed chemical method and on a serological method for horsemeat in ground meat.

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

(4) That the changes in **23.15** and **23.16** recommended in the Referee's report be adopted.

### 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 methods for added glycerol and propylene glycol in shredded coconut be studied.

(5) That chemical methods for the determination of decomposition in nuts be considered.

### MICROBIOLOGICAL METHODS

It is recommended—

(1) That work be continued on canned meats, canned acid foods, canned vegetables, eggs and egg products, nuts and nut products, frozen fruits and vegetables, sugar, and canned fishery products.

(2) That this chapter be expanded, and the value of the "Official Methods of Analysis" accordingly increased, by the inclusion of tested methods applicable to other products.

### MICROCHEMICAL METHODS

It is recommended—

(1) That the method for the micro-determination of N, as described by the Associate Referee, be further tested on compounds containing only NO and $NO_2$ groups, and that search be continued for another modification of the Kjeldahl procedure, or for a suitable pretreatment adapted to the determination of N in compounds containing the N-N linkage.

(2) That efforts to find the most suitable micro-method for sulfur be continued.

(3) That micro-methods for the determination of the halogens and phosphorus be studied.

### 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 the analysis of oils be continued.

(3) That the method proposed by the Associate Referee for the determination of propyl gallate be adopted, first action, and further studied.

(4) That studies of quantitative methods for the determination of antioxidants other than propyl gallate be continued.

(5) That the first action method for coal-tar colors in oil be adopted as official, and that further work on coal-tar colors in oil be discontinued.

### 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 no further work be done on the official method for permanganate oxidation number.

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

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

(6) That studies of methods for the determination of ash and sugars in prepared mustards be continued.

(7) That the first action method for ether extract in prepared mustard **28.31** as modified by the Referee, be made official, and that work be discontinued.

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

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

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

(11) That the method described by the Associate Referee for preparation of samples of separable types of french dressing involving the addition of albumin egg powder as emulsifier be adopted as a procedure.

(12) That french dressings of the emulsified type be prepared for sampling as in **28.38,** and that the title of this paragraph be modified so as to include them.

(13) That the method for total fat in mayonnaise and salad dressing, **28.46,** be made official and work be discontinued.

ENZYMES

It is recommended—

(1) That work on hydrocyanic glucosides be continued.

(2) 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.

(3) That the qualitative method for urea described in the Referee's report be adopted as first action.

(4) That methods for the preparation of enzymes which may not be available commercially be studied.

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## REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

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

*Malt Beverages, Brewing Materials, and Allied Products*:

It is recommended—

(1) That the study of methods for the determination of essential oils and resins in hops be continued.

(2) That the first action methods for the spectrophotometric and photometric determination of color in beer, *This Journal,* **34,** 61, (1951), be made official.

(3) That study of beer turbidity methods be continued.

(4) That methods either of removing or compensating for turbidity in samples, for color measurement, and for color in samples for turbidity measurement be studied.

(5) That methods for the degassing of beer in such a manner as to result in no change in either color or turbidity be studied.

* These recommendations submitted by Subcommittee D were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.*, 1950.

(6) That the direct, non-ash orthophenanthroline method for iron in beer, described in this year's report of the Associate Referee be adopted, first action.

(7) That the direct, non-ash orthophenanthroline procedure be further studied.

(8) That the wet-ash orthophenanthroline method be further studied and modified by the Associate Referee before submitting for collaborative study.

(9) That the first action thiocyanate procedure, **10.30, 10.31,** and **10.32,** be dropped.

(10) That the Associate Referee correlate further work on copper in beer with the work of the Subcommittee on Copper in Beer of the American Society of Brewing Chemists.

(11) That work on tin be postponed until the work on iron and copper has been 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, using the procedure described in this year's report of the Associate Referee.

(2) That the method for the determination of alcohol in wines and liqueurs using the Etienne tube (*This Journal,* **33,** 1016, (1950)) as modified in this year's report of the Associate Referee, be adopted as a procedure.

(3) That the spectrophotometric method for determining phosphates in wines and spirits as recommended by the Associate Referee be adopted, first action, and that collaborative work on it be continued.

(4) That a spectrophotometric method for the determination of tannin in wines and whiskies be studied collaboratively.

*Distilled Liquors*:

It is recommended—

(1) That the study of colorimetric methods for fusel oil be discontinued.

(2) 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).

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

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

(5) That the Williams method (*Ind. Eng. Chem.,* **18,** 841 (1926)) for determination of alcohol in distilled spirits, as modified in this year's report of the Associate Referee, be adopted as a procedure.

*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 samples of cordials and liqueurs.

### CACAO PRODUCTS

It is recommended—

(1) That the study of methods for the determination of lactose and maltose in the presence of other reducing sugars be continued.

(2) That the method for lecithin as revised in this year's report of the Associate Referee be studied collaboratively.

(3) That the method for pectic acid as given in the Referee's report for chocolate liquor, cocoa, and sweet chocolate (without milk solids), which is an editorial revision of the first action pectic acid method, **12.15,** be made official and be substituted for the first action method in **12.15.**

(4) That work on the determination of pectic acid in products containing milk solids be continued and that work on the method of conversion of pectic acid to galacturonic acid be continued.

(5) That work on characteristic cacao constituents, such as theobromine, cacao red, tannins, etc., be continued.

### CEREAL FOODS

It is recommended—

(1) That the studies 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 as reported by the Associate Referee, be adopted, first action, and study continued.

(4) That the work on the determination of proteolytic activity of flour be discontinued.

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

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

(7) That the method for water soluble protein-nitrogen precipitable by 40% alcohol (albumen) in noodle and macaroni products **13.34** be deleted and the method as reported by the Associate Referee, be adopted, first action, and study continued.

(8) That the methods for solids, **13.86,** ash **13.87,** protein **13.88,** fat

**13.89,** and crude fiber **13.90** be adopted as official for all baked products not containing fruit, and the study discontinued.

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

(10) That the method for the determination of acetic and propionic acid in bread, *This Journal,* **34,** 64–68 (1951), be adopted as official, and the study continued on other types of bread such as whole wheat, milk, etc.

(11) That Method I on nitrites, *This Journal,* **34,** 68 (1951), be adopted as official.

(12) That the method for carotene in noodles, *This Journal,* **34,** 68–69 (1951), be adopted as official.

(13) That the work on the determination of lipoid and lipoid $P_2O_5$ in noodles, as reported this year, be continued.

BAKING POWDER

It is recommended—

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

(2) That the qualitative test, Method II for Al., *This Journal,* **34,** 61 (1951), be adopted as official.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

It is recommended—

(1) That studies be made of the methods proposed in this year's report of the Associate Referee to replace those in **35.83, 35.84, 35.85, 35.86,** and **35.87.**

(2) That collaborative study be conducted on the new method for manure fragments proposed in this year's Associate Referee report.

(3) That the new method proposed in the Associate Referee's report to replace method **35.23,** be adopted, first action; and the new method for light filth in Spanish peanuts, as proposed in the Referee's report, be adopted, first action.

(4) That the seven revisions proposed in the Associate Referee's report for methods **35.2(d), 35.4(a),** (first three paragraphs), **35.28(a), 35.28(b), 35.29(a), 35.36(d),** and **35.37** be adopted, first action.

(5) That the methods for rot in apple butter, **35.48,** and for mold in frozen strawberries, **35.55,** be made official.

(6) That the methods for rot in frozen drupelet berries and the method for rot in canned drupelet berries in this year's Associate Referee's report, be made first action.

(7) That the procedure for rot fragments in tomato products described in the Associate Referee's report be made first action.

(8) That method **35.80(a)** for insect fragments in canned corn be modified as described in the Associate Referee's report and be made first action.

(9) That the error in **35.9(c)** described in the Associate Referee's report, be corrected in the manner stated and the substituted method be made first action.

(10) That the procedure for fine sediment in milk described in the Associate Referee's report be subjected to further study.

### FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the changes recommended by the Associate Referee be made in the directions for the photometric method for vanillin as given in *This Journal*, **34**, 72 (1951), and that the revised method be adopted as first action.

(2) That the changes recommended by the Associate Referee be made in the directions for the photometric method for coumarin as given in *This Journal*, **34**, 73 (1951), and that the revised method be adopted as first action.

(3) That the method for the determination of isopropyl alcohol in lemon and orange flavors as given in this year's report of the Associate Referee on organic solvents in flavors be adopted as first action.

(4) That the method for the determination of essential oil in emulsions as given in this year's report of the Associate Referee on this subject be adopted as first action.

(5) That the method for the determination of essential oil in citrus juices and other beverages as given in this year's report of the Associate Referee on this subject be adopted as first action.

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

(a) Photometric method for vanillin.
(b) Photometric method for coumarin.
(c) Method for essential oil in emulsions.
(d) Reflux method for essential oil in citrus fruit juice and other beverages.
(e) Beta-ionone where small amounts are present.
(f) Propylene glycol in vanilla extracts, *This Journal*, **33**, 103 (1950)
(g) Gravimetric method for vanillin and coumarin **19.4** and **19.5**, as applied to imitation vanilla flavors and vanilla extracts.

(7) That methods **19.6** and **19.7** be deleted, first action.

### FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the first action method for citric acid described in *This Journal*, **34**, 74–75 (1951), as corrected by the Referee, be adopted as official.

(2) That the work on the separation and determination of fruit acids be continued.

(3) That work on methods for the examination of frozen fruits and fruit products for fill of container, be continued.

(4) That work on methods for the examination of frozen fruits and fruit products for fruit, sugar, and water be continued.

### PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That collaborative study be made of the methods "Determination of Quaternary Ammonium Compounds as Reineckates" as reported by the Associate Referee.

(2) That collaborative study be made of the method "Identification of Certain Quarternary Ammonium Compounds as Reineckates," as reported by the Associate Referee.

(3) That collaborative study be continued on the following quantitative methods for the determination of quaternary ammonium compounds.

(a) Method for Fruit Juices. *This Journal*, **29**, 318 (1946).

(b) Shorter Method for Fruit Juices. *Ibid.*, **29**, 319 (1946).

(c) Method for Bottled Sodas. *Ibid.*, **29**, 323 (1946) subject to increasing the volume of bromophenol blue reagent to 5–10 ml.

(d) Method for Milk. *Ibid.*, **29**, 324 (1946) on samples containing preservative quantities of quaternary ammonium compounds.

(e) Method for Mayonnaise, Salad Dressings, and Sandwich Spreads. *Ibid.*, **29**, 323 (1946).

(f) Method for Pickles and Relishes. *Ibid.*, **29**, 326 (1946).

(g) Method for Shrimp. Ibid., **33**, 670 (1950).

(4) That the collaborative study of the method for monochloroacetic acid in beverage bases containing halogenated weighting oils, *Ibid.*, **34**, 345 (1951), be continued.

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

(6) That study of the method for thiourea be continued.

(7) That the method reported by the Associate Referee be adopted, first action, for the detection of propoxy-2-amino 4 nitrobenzene (P-4000).

(8) That the method reported by the Associate Referee be adopted, first action, for the detection of cyclohexylsulfamate (sucaryl sodium).

(9) That **27.10** be revised as reported by the Associate Referee, and made official.

(10) That quantitative methods for the determination of dulcin, sucaryl sodium, and P-4000 be studied.

(11) That the official etching methods for fluorides, **27.11, 27.12, 27.13,** and **27.14,** be studied collaboratively, in comparison with the Gettler method (which depends on the formation of hexagonal pink crystals of sodium fluosilicate).

(12) That an Associate Referee be appointed to study methods for the detection and determination of benzoates and hydroxybenzoates.

### 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 sugar 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,** incl., be continued.

(6) That until a satisfactorily rapid method for the determination of color and turbidity in unfiltered solutions is developed, the color of sugar products be determined at wave length 560 m$\mu$ by the method given in this year's report of the Associate Referee, with celite analytical filter aid, and expressed as absorbancy index.

(7) That the study of the transmittancy of sugar solutions be continued, by determining the precision of the method, having each collaborator make at least three complete replicate experiments with the same raw sugar, all collaborators agreeing to carry out the work at a predetermined period of time.

(8) That the Folin and Wu micro methods, *Biol. Chem.* **41,** 367 (1920), for the determination of dextrose be adopted, first action.

(9) That the refractive index values for raffinose hydrate solutions, reported by Zerban and Martin at the 1951 meeting of the A.O.A.C., be adopted.

### 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-FIFTH ANNUAL MEETING, OCTOBER 1, 2, AND 3, 1951*

The changes recorded in the methods of the Association, as given below, become effective, as provided in Sec. 8 of the by-laws, on the thirtieth day from the date of publication of this Report, Feb. 15, 1952.

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 first-action method for water-soluble magnesium in other materials including mixed fertilizers, **2.57(b)** (p. 23) was adopted as official.

(2) The official citrate-insoluble phosphoric acid method, **2.16** and **2.17** (p. 10), using continuous agitation during digestion was adopted as applicable to calcium metaphosphate on samples ground to pass a 35 mesh sieve.

(3) The following wet-digestion method for potash was adopted, first action:

### REAGENTS

(a) *Platinum soln.*—Use a Pt soln. containing the equivalent of 0.5 g of Pt (1.05 g $H_2PtCl_6$) in every 10 ml.

(b) *Diglycol stearate soln.*—Dissolve 20 g of diglycol stearate (tech.) in 1 liter of equal parts of benzene and ethyl alcohol.

### PREPARATION OF SOLN.

Place 2.5 g or the factor weight 2.425 g of a sample in a 250 ml volumetric flask. Add 125 ml of $H_2O$, 50 ml of saturated $NH_4$ oxalate soln, and 1 ml of diglycol stearate soln when necessary to prevent foaming. Boil 30 min, add slight excess of $NH_4OH$ and, after cooling, dilute to 250 ml. Mix and pass thru dry filter.

### DETERMINATION[1]

Place a 50 ml aliquot of soln (or a 25 ml aliquot and 25 ml $H_2O$, if sample contains over 20% $K_2O$) in a 500 ml Kjeldahl flask. Add 10 ml $HNO_3$ and a silica granule (about 1 cm long) previously weighed along with a prepared Gooch or medium fritted crucible (Pyrex M porosity). Boil 2 min and add 10 ml HCl. Boil down to ca 25 ml and add 5 ml HCl and excess Pt soln. Boil down to 10–15 ml, rotating flask occasionally, and then add 5 ml HCl. Reduce heat and boil down to 3–5 ml (depending on amount of precipitate), rotating flask frequently near the end of the evaporation. Remove flask from heat and swirl to dissolve any soluble residue on walls. After cooling, immediately add 25 ml of 95% alcohol so that it washes down neck of

* Unless otherwise given all references in this Report are to *Methods of Analysis, A.O.A.C.*, 1950.

[1] Perrin, *Anal. Chem.*, 21, 984 (1949), slightly modified.

flask. Chill under tap, swirl and allow to stand for at least 5 min. Decant into the tared crucible and transfer precipitate and granule with the aid of a stream of 95% alcohol. Wash 5–6 times with 10 ml portions of $NH_4Cl$ soln **(2.39(a))** to remove magnesium and sodium salts from ppt. Wash again thoroly with alcohol and dry ppt. for 30 min at 100°C. Weigh and subtract weight of crucible plus the silica granule. $K_2PtCl_6 \times 0.19376 = K_2O$.

## 3. SOILS

(1) The following neutral calcium acetate method for the replacement of the exchangeable hydrogen of soils and determination thereof was adopted, first action:

### REAGENTS

**(a)** 0.5 *M Ca-acetate.*—Prepare 0.5 *M* calcium acetate by dissolving 176.1 g $Ca(C_2H_3O_2)_2 \cdot H_2O$ in 2 liters of $H_2O$; adjust to *p*H 7 by titration with 1 *N* acetic acid, using the glass electrode as indicator. (Ca 4 ml of the acid will be required per 2 liters.) Make supply needed for a week at a time, store in bottle provided with siphon and soda-lime tube.

**(b)** 0.1 *N* $Ba(OH)_2$—Dissolve 1/20 molar wt of $Ba(OH)_2 \cdot 8H_2O$, C.P. per liter, allowing a 2% excess for impurities and $CO_2$ contamination. Let stand 24 hours. Prepare a storage bottle connected with syphon and soda-lime guard tube and freed of $CO_2$. Draw $Ba(OH)_2$ soln into the prepared bottle by applying suction at the soda-lime tube and drawing the soln thru a sintered glass filter. Connect the $Ba(OH)_2$ soln with a 25 ml pinch cock burette, provided with side tube for gravity feed, and a soda-lime tube at the top.

### DETERMINATION

Weigh soil charges calculated to contain 1–2 m.e. of exchangeable hydrogen; place in 250 ml Erlenmeyer flasks; introduce ca 100 ml of the Ca-acetate soln; stopper and shake several times during first hour. Let stand overnight. Filter soil suspension thru 12.5 cm filter papers, placed on 3-inch funnels resting upon 250 ml volumetric flasks. Transfer soil onto filter by the aid of stream of the soln; wash the soil with small quantities of the soln until the volume is just below the 250 ml mark. Remove funnel, discard soil, and make filtrate to volume with same soln. Transfer to 400 ml beaker; rinse flask with $H_2O$ and titrate potentiometrically with $Ba(OH)_2$ to *p*H 8.8. Obtain also the titration value of 250 ml of the replacing soln. Calculate the exchangeable hydrogen in m.e. per 100 g of soil from the formula: $((Tu\text{-}Tb)/Wt.) \times 10$, in which—

Tb equals titration value of replacing soln;

Tu equals titration value of soil extract, both expressed in ml of 0.1 *N* $Ba(OH)_2$;

Wt. equals weight of soil charge.

(2) The first action method for fluorine, *This Journal*, **34**, 58 (1951), reworded by the Associate Referee as follows, was adopted as official:

### REAGENTS

**(a)** *Sulfuric acid.*—1+1 soln.

**(b)** *Sodium hydroxide.*—10% soln.

**(c)** *Sodium hydroxide.*—.05% soln.

**(d)** *Perchloric acid.*—70% reagent containing 0.2% of $Ag_2SO_4$ (6g $Ag_2SO_4$ dissolved in 7 lb bottle of $HClO_4$).

**(e)** *Phenolphthalein.*—0.1% soln in 1+1 alcohol.

**(f)** *Sodium alizarin sulfonate.*—0.05% aqueous soln.

(g) *Buffer.*—Dissolve 2 g NaOH in 50 ml $H_2O$. Add this to aqueous soln of 9.44 g monochloroacetic acid and dilute total to 100 ml.

(h) *Thorium nitrate soln.*—0.01 *N*. Dissolve 1.38 g of $Th(NO_3)_4 \cdot 4H_2O$ in $H_2O$, dilute to 1 liter, and standardize as directed in **5.21(b)**.

(i) *Thorium nitrate soln.*—0.02 *N*. Dissolve 2.761 g of $Th(NO_3)_4 \cdot 4H_2O$ in $H_2O$, dilute to 1 liter, and standardize as directed in **5.21(b)**.

(j) *Sodium fluoride standard.*—2.21 g NaF per liter.

DETERMINATION

Transfer a 0.5 g charge of 100 mesh soil into 125 ml Claisen distn flask. Provide flask with thermometer and steam inlet tube, connect to condenser (apparatus as shown in **5.21(b)**) and add 50 ml 1+1 $H_2SO_4$ to charge. Apply heat and raise temp. to 150°. Introduce steam current, then raise temp. to 165° and at that temp. steam distill to collect 500 ml at the rate of about 4 ml per min. Make collection in 600 ml beaker and keep distillate alkaline to phenolphthalein by addition of the 10% NaOH as required.

Evaporate distillate to 10–15 ml and transfer to Claisen flask, rinsing beaker with 15 ml of the 70% $HClO_4$ containing 0.2% $Ag_2SO_4$, and add 10 ml additional.

Connect flask to distn apparatus, raise temp. to 130±5°, introduce steam current and, with temp. maintained, distill just under 200 ml into 250 ml wide-mouth Erlenmeyer flask, at rate of 4 ml per min. Adjust volume to exactly 200 ml in volumetric flask.

Transfer 100 ml aliquot of distillate into 150 ml beaker. Add 2 ml Alizarin indicator; neutralize soln to faint pink with 0.05 *N* NaOH, then adjust to *p*H 3.0 by addition of 1 ml of the buffer. Titrate to a faint pink end point by addition of 0.02 *N* Thorium nitrate from micro-burette with 0.01 ml calibrations. The reading should be between 0.5 and 1.0 ml, according to the fluorine content of sample; if appreciably higher reading is obtained, repeat titration with smaller aliquot (50 or 25 ml) diluted to 100 ml; if reading was too low, repeat titration on 100 ml aliquot, using the weaker Thorium nitrate soln (0.01 *N*). In any event, make duplicate titrations. Calculate the fluorine equivalence of the titration from the standard curve and express results as p.p.m. of F.

## 4. CAUSTIC POISONS

No additions, deletions, or other changes.

## 5. ECONOMIC POISONS

(1) The following method for the determination of potassium cyanate in herbicides was adopted, first action:

REAGENTS

(a) *Semicarbazide hydrochloride,* $NH_2CONHNH_2 \cdot HCl$.

(b) *Wash soln* (water saturated with hydrazodicarbamide).—Prepare by mixing some potassium cyanate and semicarbazide hydrochloride in water, filter and wash the precipitate with water. Transfer the precipitate to a flask, add small quantity water, shake vigorously, and filter. (The solubility of the precipitate in water is about one part in 6600.)

DETERMINATION

Transfer a sample containing 0.2–0.5 g potassium cyanate to a 100 ml beaker, add 20 ml wash soln and 1 g semicarbazide hydrochloride ($NH_2CONHNH_2 \cdot HCl$), and allow to stand 24 hours. Filter on a Gooch or Grade F sintered crucible, wash with 10 ml of the wash soln **(b)**, and dry at 100°C. to constant weight. (The factor

from the precipitated hydrazodicarbamide ($NH_2CONHNHCONH_2$) to potassium cyanate is 0.68682.)

(2) The following revisions were made in the official method for pyrethrin I, **5.111** (p. 72), and adopted as first action.

Par. 2, line 6, beginning with "Do not shake before making to vol.," delete thru " . . . two 50 ml portions of petr. ether.," and substitute the following:

"Swirl gently, allow the precipitate to settle, and test the supernatant liquid with a little more $BaCl_2$ soln to see if sufficient has been added to obtain a clear soln. Do not shake before making to vol. Make to vol., mix thoroly, and filter off 200 ml thru a fluted 12.5 cm filter paper, and transfer to a 500 ml separator. Add 2–3 drops of phenolphthalein indicator, **2.11(d)**, neutralize with HCl (conc.) and add 1 ml in excess. Add 5 ml of satd NaCl soln and extract with two 50 ml and one 25 ml portions of petr. ether."

(3) The following revisions were made in the first action method for Pyrethrin II, **5.112** (p. 73), and adopted as first action:

Beginning with "conc. filtrate to ca 50 ml, . . . . " delete thru " . . . and dry residue at 100° 10 min.," and substitute the following:

"Conc. filtrate to ca 50 ml, transfer to 500 ml separator. Acidify with 10 ml HCl (conc.) and sat. with NaCl. (Care must be taken that the acidified aq. layer is satd with NaCl thruout following extns.) Extract with 50 ml of ether, draw off aq. layer into 2nd separator and extract again with 50 ml of ether. Continue extn and drawing off of aq. layer, using 35 ml for 3rd and 4th extns. Combine 4 ether exts, drain, and wash with three 10 ml portions of satd NaCl soln. Filter ether exts thru plug of cotton into 500 ml Erlenmeyer flask, and wash cotton with addnl 10 ml of ether. Evap. ether on $H_2O$ bath, and remove any fumes of HCl with current of air and continued heating. Dry in an oven at 100° for 10 min."

(4) The following revisions were made in the first action method for Pyrethrum Extracts in Mineral Oil, Pyrethrin I, **5.114** (p. 74), and adopted, first action:

Beginning with "To aq. soln in 250 ml. flask, . . . " delete thru " . . . Filter thru 7 cm filter paper." and substitute the following:

"To aq. soln in 250 ml flask, add 1 g of Filter Cel and 10 ml of the $BaCl_2$ soln. Swirl gently, allow the precipitate to settle, and test the supernatant liquid with a little more $BaCl_2$ soln to see if sufficient has been added to obtain a clear soln. Do not shake before making to vol. Make to vol., mix thoroly, and filter off 200 ml thru a fluted 12.5 cm filter paper, and transfer to a 500 ml separator. Add 2–3 drops of phenolphthalein indicator, **2.11(d)**, neutralize with HCl (conc.) and add 1 ml in excess. Add 5 ml of satd NaCl soln and ext. with two 50 ml and one 25 ml portions of petr. ether. Then proceed as directed under **5.111**, par. 2, beginning "Wash exts with 2 or 3 10 ml portions of $H_2O$."

(5) The following methods for technical parathion and dust formulations were adopted, first action:

APPARATUS

Photoelectric colorimeter, equipped with a filter to give maximum transmission between 400 and 450 millimicrons.

### REAGENTS

(1) *Ethyl ether, C. P.*

(2) *Sodium carbonate,* 1%

(3) *Sodium hydroxide,* 1*N*

(4) *Acetic acid-hydrochloric acid,* 9 volumes of glacial acetic acid in 1 volume concentrated hydrochloric acid.

(5) *Sodium (or potassium) bromide, C. P.*

(6) *Zinc dust,* iron-free

(7) *Potassium iodide-starch paper*

(8) *Sulfanilic acid,* Eastman Kodak

(9) *Sodium nitrite soln,* 0.1 *N,* standardized against anhydrous recrystallized sulfanilic acid. (Restandardize weekly).

### SEPARATION OF PARATHION AND *p*-NITROPHENOL

Weigh accurately from a weighing pipet 0.6–0.9 g of sample into 100 ml of ethyl ether contained in a 250 ml separatory funnel. Extract the ether soln four times (or until the extract is colorless) with 20 ml portions of chilled, 1% sodium carbonate soln, collecting the combined aqueous layers in a 200 ml volumetric flask. Transfer the ether layer to a 400 ml beaker, using small portions of ether to effect the quantitative transfer.

### DETERMINATION OF *p*-NITROPHENOL IN AQUEOUS EXTRACT

*Preparation of Standard Curve.*—Weigh accurately 100 mg of *p*-nitrophenol, transfer to a 1 liter volumetric flask, and make up to volume with 0.1 *N* sodium hydroxide. Transfer 2, 4, 6, 8, 10, and 20 ml aliquots of this soln to 100 ml volumetric flasks, and make each soln up to volume with 0.1 *N* sodium hydroxide. Read the light transmittancy of each of the standard solns at 400 mμ by means of a photoelectric colorimeter which has been set to give 100% transmittance with water. Plot the light transmittancies as abscissas against the concentrations (milligrams per milliliter) as ordinates on semilogarithmic graph paper.

*Determination of p-Nitrophenol.*—Add 20 ml of 1 *N* sodium hydroxide to the combined aqueous extracts contained in the 200 ml volumetric flask and make up to volume with water. Measure the light transmittancy of the soln at 400 mμ with a photoelectric colorimeter and read from the standard curve the concentration of *p*-nitrophenol in milligrams per milliliter of solution.

$$\% \text{ } p\text{-nitrophenol} = \frac{\text{mg/ml} \times 200 \times 100}{1000 \times \text{grams of sample}}$$

A dilution of the sodium *p*-nitrophenoxide soln to 200 ml is suitable for technical parathion containing up to 0.2% *p*-nitrophenol. If the sample being analyzed contains greater amounts of *p*-nitrophenol, the sodium *p*-nitrophenoxide soln must be diluted with 0.1 *N* sodium hydroxide to bring the concentration of sodium *p*-nitrophenoxide in the soln to be measured within the limits of 0.003 to 0.010 mg per ml. The dilution necessary will vary with the sample.

### DETERMINATION OF PARATHION IN ETHER SOLUTIONS

*Standardization of sodium nitrite.*—Weigh accurately 0.4–0.45 g of anhydrous recrystallized sulfanilic acid (the purity of which has been checked by a nitrogen determination) into a 400 ml tall-form beaker. Add 80 ml of water, 10 ml of concentrated hydrochloric acid, 30 ml of glacial acetic acid, and 5 g of sodium bromide. Cool the mixture to 0°–10°C. by the addition of clean shaved ice and place under mechanical stirring. Titrate at 0°–10°C. with 0.1 *N* sodium nitrite as rapidly as the spot test will permit. Near the end point add the nitrite in 4 drop portions.

*Spot test.*—Dip a glass rod into the soln to be tested and then touch the rod quickly to a piece of potassium iodide-starch paper. The end point is reached when an intense blue-black color appears immediately and can be obtained repeatedly during a 1 min. period without further addition of the nitrite.

$$\text{Normality of sodium nitrite} = \frac{\text{grams of sulfanilic acid} \times 1000}{\text{ml of } NaNO_2 \times 173.2}$$

*Analysis of ether solution for parathion.*—Add 35 ml of the acetic-hydrochloric acid soln to the ether soln of parathion which has been transferred to a 400 ml beaker. Add 1 g of zinc dust and cover the beaker with a watch glass. Heat the soln gently on a steam bath until most of the ether has evaporated and the soln is colorless. Add 10 ml of concentrated hydrochloric acid to complete solution of the zinc dust. Cool the soln, wash down the beaker and watch glass with 100 ml of water, and add 5 g of sodium (or potassium) bromide. Cool the mixture to 0°–10°C. by the addition of clean shaved ice (ca 100 g) and place under mechanical stirring. Titrate at 0°–10°C. with standardized 0.1 *N* sodium nitrite as rapidly as the spot test will permit. (About 20–30 ml will be required.) Near the end point add the nitrite in 4 drop portions.

$$\%\ \text{parathion} = \frac{\text{ml of sodium nitrite} \times \text{normality of sodium nitrite} \times 29.13}{\text{grams of sample}}$$

ANALYSIS OF DUST PREPARATIONS

Transfer a weighed sample of dust or wettable powder to an extraction thimble and extract with 150 ml of ethyl ether in a Soxhlet apparatus for 1 hour. Transfer the ether extract to a 250 ml separatory funnel and proceed with the analysis as described above.

The sample size will vary with the concentration of the dust:

10% = 6.75 grams; 15% = 4 to 5 grams; 25% = 2.5 to 3.5 grams.

## 6. PLANTS

No additions, deletions, or other changes.

## 7. BAKING POWDERS

(1) The first action method for the qualitative test for aluminum (*Method II*), *This Journal*, **34**, 61 (1951), was adopted as official.

## 8. BEVERAGES: NON-ALCOHOLIC AND CONCENTRATES

No additions, deletions, or other changes.

## 9. BEVERAGES: DISTILLED LIQUORS

(1) The spectrophotometric method for phosphates in wines and spirits given under *Changes in Methods*, Chapter 11, item (2), was adopted, first action.

(2) The following Williams field test method for alcohol was adopted as a procedure:

ALCOHOL (WILLIAMS FIELD TEST METHOD)[1]

APPARATUS

*Williams Tube.*—Clean frequently with a synthetic detergent using a small fiber brush. Dry.

[1] *Ind. Eng. Chem.*, **18**, 841 (1926).

### REAGENT

*Solvent.*—Mix 70 ml pentasol (synthetic amyl alcohol), 28 ml toluene, and 2 ml 10% HCl. Shake well until acid is completely dissolved.

### METHOD

Introduce 10 ml spirits into the tube. Accurately adjust the bottom of the meniscus to coincide with the 7.5 ml mark. Remove any excess sample on the sides of the tube above the 7.5 ml mark by means of a swab or roll of filter paper. Introduce 7.5 ml reagent to the 15 ml mark. Stopper the tube and invert a number of times to insure intimate mixing. Stand the tube in an upright position and allow to separate. When separation is complete shake down globules of lower soln that adhere to sides and stopper, by flipping and twirling the tube between thumb and finger. When settling and drainage are complete, read the percentage of alcohol where the meniscus between the two layers falls on the calibration marks. Repeat the above operation of mixing and settling and read again.

### TEMPERATURE CORRECTION

For more consistent results a correction may be made for the effect of temperature and the variations caused by differing percentages of alcohol present. These correction factors are given in the table and are applied as follows:

Indicated percentage of alcohol from tube, 48.0% at 90°F.

Correction factor from the table is −1.1; 48.0% − 1.1 = 46.9%

*Temperature correction factors*

| % | 60° | 62° | 64° | 66° | 68° | 70° | 72° | 74° | 76° | 78° |
|---|---|---|---|---|---|---|---|---|---|---|
| 43 | + .5 | + .3 | + .2 | 0.0 | − .1 | − .2 | − .4 | − .5 | − .7 | − .8 |
| 43.4 | + .5 | + .4 | + .2 | + .1 | 0.0 | − .2 | − .3 | − .5 | − .6 | − .7 |
| 44 | + .6 | + .4 | + .3 | + .2 | 0.0 | − .1 | − .3 | − .4 | − .5 | − .7 |
| 45 | + .7 | + .5 | + .4 | + .3 | + .1 | 0.0 | − .2 | − .3 | − .4 | − .6 |
| 46 | + .8 | + .7 | + .5 | + .4 | + .2 | + .1 | 0.0 | − .2 | − .3 | − .5 |
| 47 | + .9 | + .8 | + .6 | + .5 | + .3 | + .2 | + .1 | − .1 | − .2 | − .4 |
| 47.5 | +1.0 | + .8 | + .7 | + .5 | + .4 | + .3 | + .1 | 0.0 | − .2 | − .3 |
| 48 | +1.1 | + .9 | + .7 | + .6 | + .4 | + .3 | + .2 | 0.0 | − .1 | − .2 |
| 49 | +1.1 | +1.0 | + .8 | + .7 | + .6 | + .4 | + .3 | + .1 | 0.0 | − .1 |
| 50 | +1.2 | +1.1 | + .9 | + .8 | + .7 | + .5 | + .4 | + .3 | + .1 | 0.0 |

| % | 80° | 82° | 84° | 86° | 88° | 90° | 92° | 94° | 96° | 98° |
|---|---|---|---|---|---|---|---|---|---|---|
| 43 | − .9 | −1.1 | −1.2 | −1.4 | −1.5 | −1.6 | −1.8 | −1.9 | −2.0 | −2.2 |
| 43.4 | − .9 | −1.0 | −1.2 | −1.3 | −1.4 | −1.6 | −1.7 | −1.9 | −2.0 | −2.2 |
| 44 | − .8 | − .9 | −1.1 | −1.2 | −1.4 | −1.5 | −1.6 | −1.8 | −1.9 | −2.1 |
| 45 | − .7 | − .8 | −1.0 | −1.1 | −1.3 | −1.4 | −1.5 | −1.7 | −1.8 | −2.0 |
| 46 | − .6 | − .7 | − .9 | −1.0 | −1.2 | −1.3 | −1.4 | −1.6 | −1.7 | −1.8 |
| 47 | − .5 | − .6 | − .8 | − .9 | −1.0 | −1.2 | −1.3 | −1.5 | −1.6 | −1.7 |
| 47.5 | − .4 | − .6 | − .7 | − .8 | −1.0 | −1.1 | −1.3 | −1.4 | −1.5 | −1.7 |
| 48 | − .4 | − .5 | − .6 | − .8 | − .9 | −1.1 | −1.2 | −1.3 | −1.5 | −1.6 |
| 49 | − .3 | − .4 | − .5 | − .7 | − .8 | −1.0 | −1.1 | −1.2 | −1.4 | −1.5 |
| 50 | − .2 | − .3 | − .4 | − .6 | − .7 | − .9 | −1.0 | −1.1 | −1.3 | −1.4 |

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

(1) The first action methods for the spectrophotometric and photometric determination of color in beer, *This Journal*, **34**, 61–63 (1951), were adopted as official.

(2) The following direct, non-ash orthophenanthroline method for iron in beer was adopted, first action:

### PHOTOMETER

Any of the commercial types of photometers will be suitable for this determination. Use a filter or wave length peak in the blue-green region (500–550 mμ) or more specifically, 505–520 mμ.

### REAGENTS

**(1)** *Standard iron soln.*—(1 ml = 0.1 mg Fe or 1.00 p.p.m. Fe in 100 ml)

**(a)** *Iron wire.*—Dissolve 0.500 g of reagent grade iron wire, wiped free of oxide, in 5 ml of 20% HCl plus 1 ml of conc. $HNO_3$. Cover with a watch glass, heat and evaporate to dryness; add distilled water and evaporate to dryness again. Take up the residue with 3–5 ml of conc. HCl, cool, and transfer quantitively to a 500 ml volumetric flask. Add 2 drops of bromine water and dilute to volume and mix. This is soln A. Transfer 50 ml of soln A and 2 drops of bromine water to a 500 ml volumetric flask and dilute to volume with distilled water. This is soln B.

**(b)** *Ferrous ammonium sulfate hexahydrate* ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$).—Dissolve 3.512 g of the above salt in distilled water, add 5 ml conc. hydrochloric acid, and dilute to 500 ml. This is soln A. Transfer 50 ml of soln A to a 500 ml volumetric flask and dilute to volume with distilled water. This is soln B.

**(2)** *Hydroxylamine hydrochloride* (10%).—Dissolve 100 g of hydroxylamine hydrochloride in 1000 ml of distilled water.

**(3)** *O-phenanthroline* (0.3%).—Dissolve 1.5 g of *o*-phenanthroline monohydrate in 500 ml distilled water heated to not more than 80°C.

### *Calibration of Photometer*

Depending upon the size of the photometer cell to be used, prepare a series of 25 or 50 ml *beer standards* containing 0, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 p.p.m. iron. Develop the color in these standards according to the procedure outlined below. Transmission values may be read, and plotted against p.p.m. Fe on semi-log paper, or they may be converted to corresponding optical density values, or optical densities may be read directly and the data used to calculate a suitable factor converting densities to p.p.m. Fe.

### PROCEDURE

Depending on the size of photometer cell to be used, pipet two aliquots of 25 or 50 ml of degassed beer to 125 ml Erlenmeyer flasks or 150 ml beakers. Add 1 ml of the hydroxylamine hydrochloride reagent to each, cover, and allow to stand for 30 min. Then to one add 2 ml of the *o*-phenanthroline reagent and to the other 2 ml of distilled water. After 30 min. compare the solns in the photometer against distilled water at a wave length of 505 to 520 mμ (or in the blue-green region 500 to 550 mμ).

At the same time read the colored solution of the sample with the blank, instead of distilled water, set at 100% transmission.

### CALCULATIONS

**(1)** If transmission values are read, and

(a) Distilled water is used as the reference, the iron values of the sample and the blank are read directly from the standard curve and the difference is the p.p.m. of iron in the beer. (p.p.m. Fe. in sample) − (p.p.m. Fe in blank) = p.p.m. Fe in beer, or

(b) The blank is used as the reference, the iron value of the sample is read directly from the standard curve and is the p.p.m. of iron in the beer. p.p.m. Fe in sample = p.p.m. Fe in beer.

(2) If optical density values are read or converted from transmission values, and

(a) Distilled water is used as the reference, the difference between the optical density values multiplied by a calculated factor will give p.p.m. of iron in the beer.

(O.D. Sample − O.D. blank) × factor = p.p.m. Fe in the beer,

or

(b) The blank is used as the reference, the optical density of the sample multiplied by a calculated factor will give p.p.m. of iron in the beer.

(3) The first action method for iron in beer, **10.30–10.32** (p. 146), was deleted.

## 11. BEVERAGES: WINES

(1) The following method for the determination of alcohol in wines using the Etienne tube, *This Journal*, **33**, 1016 (1950), was adopted as a procedure.

### APPARATUS

(a) *Etienne tube.*—Clean frequently with a synthetic detergent and a small bristle brush. Dry.

### REAGENT

(a) *Solvent.*—Mix 70 ml Pentasol (synthetic amyl alcohol), 28 ml toluene and 1.8 ml HCl 10%. Shake well until acid is completely dissolved.

(b) *Carbon.*—Activated carbon (Darco S-51.)

### PREPARATION OF SAMPLE

(a) *Carbon treatment.*—Place 30–40 ml of wine into any convenient size flask and add sufficient carbon (about 0.5 g) to fairly well decolorize the wine. Stopper and shake for about 30 seconds. Pour the entire contents onto a dry filter paper. If first portions of filtrate contain suspended carbon, refilter until filtrate is clear. (Small traces of carbon do not interfere. This volume provides sufficient sample for rinsing and duplicate tests if desired.)

### DETERMINATION

Introduce 10 ml of the wine sample, straight or prepared as given above, into the tube with a pipette. Accurately adjust the bottom of the meniscus to coincide with the 10 ml mark. Remove any excess sample on the sides of the tube above the 10 ml mark by means of a swab or roll of filter paper. Introduce 20 ml of reagent to the 30 ml mark. Stopper the tube with a close-fitting rubber stopper and invert the tube a number of times with moderate shaking to insure an intimate mixing of the solns (ca two min.) Stand the tube in an upright position and allow separation to take place. When separation is complete shake down globules of the lower soln that adhere to the sides of the upper tube and stopper, by flipping and twirling the tube between the thumb and fingers. When settling and drainage is complete, usually five min., read the percentage of alcohol where the meniscus between the two layers falls on the calibration marks. Repeat the above operation of mixing and settling and read again.

TEMPERATURE CORRECTION

A correction can be made for the effect of temp., as follows: 0.07% alcohol is added to or subtracted from the observed percentage, for each degree above or below 78°F. When the temperature is above 78°F. the correction is subtracted, and when the temperature is below 78°F. the correction is added.

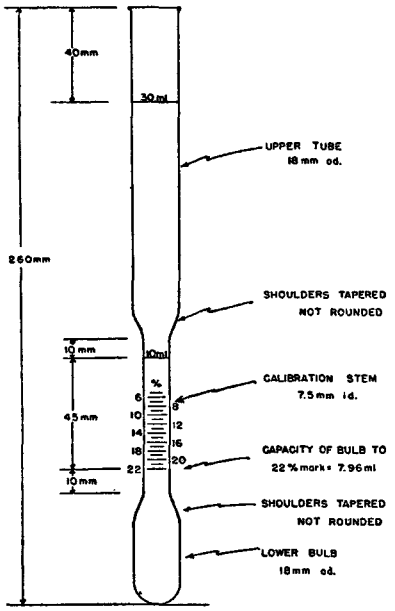


FIG. 1.—Etienne tube.

(2) The following method for phosphates in wines and spirits was adopted, first action:

REAGENTS

*Sulfuric acid.*—10 *N.*

*Sulfuric acid.*—0.1 *N.*

*Molybdate reagent.*—25 g of ammonium molybdate and 500 ml of 10 Normal sulfuric acid made to 1 liter.

*Bisulfite soln.*—150 g of sodium hydrogen sulfite per liter of soln. Let stand two days and filter. Keep well stoppered.

*Sulfite soln.*—200 g of anhydrous sodium sulfite per liter of soln. Filter if necessary and keep well stoppered.

*Sulfonic acid reagent.*—1.25 g of pulverized 1, 2, 4-aminonaphtholsulfonic acid to 490 ml bisulfite soln. After shaking, sulfite soln is added in 5 ml portions until a clear soln results, normally requiring 25 ml sulfite. This reagent should be checked against known phosphate samples at intervals of about one week.

*Standard phosphate.*—0.4389 g of reagent grade anhydrous potassium dihydrogen phosphate per liter of soln. (1 ml contains 0.1 mg P or 0.229 mg $P_2O_5$.)

DETERMINATION

Pipette 10 ml of wine or spirits into a platinum dish and evaporate to dryness in a 100°C. oven. Carefully char over a low flame to avoid decrepitation, and ash in a muffle furnace at a temp. not to exceed 550°. Dissolve the ash with 10 ml of 0.1 *N* sulfuric acid, transfer quantitatively to a 100 ml volumetric flask and dilute to mark.

Pipette 25 ml of the above soln into a 100 ml volumetric flask, add 50 ml of water, 10 ml of molybdate reagent, and 4 ml of sulfonic acid reagent, shaking after each addition, and dilute to mark. Determine the transmittancy exactly ten min. after the addition of the sulfonic acid reagent at 830 mμ. Determine P from standard curve prepared from 0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml standard phosphate soln diluted with water to ca 65 ml in a 100 ml volumetric reagent, and 4 ml sulfonic acid reagent. Shake after each addition and proceed as above. The temp. of the sample may be ±3°C. from the temp. at which the calibration curve was determined without introducing appreciable error.

## 12. CACAO BEAN AND ITS PRODUCTS

(1) The first action method for pectic acid **12.15 (a)** and **(b)** (p. 180), as revised editorially by the Referee,* was adopted as official:

**(a)** *In sweet chocolate containing no milk solids.*[1]—(1) *Extraction of fat*—Weigh, within ±0.15 g, such quantity (14–60 g) of grated and well mixed sample as will contain 4.7–5.2 g of fat-free dry cacao, and place in one, or two, 250 ml centrifuge bottles. (If sample exceeds 50 g, distribute it about equally between the 2 bottles: Make detns. in duplicate.) Add 120 ml of petr. ether (B. P. 30°–65°), or ether, at about 30°, to each bottle, shake thoroly, centrifuge, and decant supernatant liquid. Repeat extn. with another 100 ml of solvent, then ext. with 100 ml of 95% alcohol, decant, and discard exts.

(2) *Extraction of color, tannins, etc.*—Add to each bottle (using graduate) 150 ml of acidified 82% alcohol (10 ml of HCl plus 432 ml of 95% alcohol dild. to 500 ml with $H_2O$) that has been warmed so that the temp. of the liquid in the centrifuge bottle is 55°C. Stopper, shake vigorously 2 min., centrifuge 6–8 min., decant, and discard supernatant liquids. Add 100 ml of alcohol (95%) to residue in each bottle, shake, centrifuge as before, and decant and discard exts.

(3) *Extraction of pectin.*—Measure out 150 ml of $H_2O$ in graduate, add ca 75 ml to one bottle, stopper, shake vigorously to disperse residue thoroly, decant into other bottle containing remainder of sample, and again shake vigorously until residue is thoroly dispersed. Decant mixt. into 500 ml wide-mouthed Erlenmeyer flask, rinse mouth of bottle with small squirt of $H_2O$ (ca 1 ml) from wash bottle, and complete transfer of residue from bottles with about 45 and 30 ml successive portions of $H_2O$ remaining in graduate. Make mixt. in flask just alk. to litmus with $NH_4OH$ (1 plus 1) (about 0.7 ml: note quantity used: see note[2]). Acidify with acetic acid, add 0.5 ml in excess, and then add 50 ml of 2% weight/volume $(NH_4)_2C_2O_4 \cdot H_2O$ soln, using soln to wash down sides of flask.

Pass glass stirrer (with 1–1½″ diam. loop, perpendicular to shaft, on end) thru glass tube of slightly larger diam. held in rubber stopper placed in mouth of flask. Attach shaft of stirrer to motor, or air rotor, that will stir contents of flask continuously, immerse flask below level of contents in $H_2O$ bath maintained at 90–92°,

* For the convenience of the reader, 12.15(a) and (b) as editorially changed, are herewith reprinted in full.

[1] Sweet chocolate is usually characterized by its color. Analysts should note that small quantities of milk solids may be present, and this point should be kept in mind. If in doubt, use method for milk chocolate, 12.15(c).

[2] For note referred to see p. 183, *Methods of Analysis.*

and stir moderately 3 hrs. (If level of liquid in flask is appreciably reduced, add sufficient hot $H_2O$ to bring back to original level.)

Remove flask, cool to 45°, transfer contents quantitatively to 250 ml volumetric flask, dil. to vol. with $H_2O$ at 45°, and add 1.5 ml excess to correct for vol. of cacao solids. Mix contents well, pour into centrifuge bottle, and whirl at 1800 r.p.m. about 15 min. Decant supernatant ext., which may be turbid or opalescent, into 400 ml beaker. Rinse any residue in flask into centrifuge bottle with 95% alcohol and reserve this cacao residue for further treatment to estimate fat-free cacao in sample. Warm ext. to 45°, pour into graduate, note vol., and return to beaker. Rinse graduate with two 5 ml portions of $H_2O$, and add to beaker. Cool in bath to 15–17°, make alk. to phenolphthalein (internal indicator) with 15% NaOH soln, and add 11 ml in excess. (See note.[2]) Stir, and allow to stand in the bath at 15–17° for 20 min. Decant alk. liquid into two 250 ml centrifuge bottles, distributing vol. about equally. Let drain, and rinse twice with 5–8 ml of cold $H_2O$, adding one rinsing to each bottle. Add to each bottle, with stirring, 10 ml of HCl, and then add gradually with continued stirring 40 ml of 95% alcohol. Add to each bottle 0.8–1.0 g of 1:1 mixt. of Filter Cel and Celite 545, stir, rinse off rod, stopper bottles, shake well, and centrifuge 10–12 min. Decant and discard supernatant liquids without disturbing sediment, and wash residues once by shaking contents of each bottle with 100 ml of 95% alcohol, centrifuging, and decanting supernatant liquids.

Add 75 ml of $H_2O$ to one bottle, stopper, and shake well. Make slightly alk. with a few drops to $NH_4OH$ (1 plus 1) and shake again. Decant liquid into 2nd bottle, stopper, shake again, make alk. to litmus with $NH_4OH$ (1 plus 1), and add 0.5 ml in excess. Stopper, and shake thoroly 1–1½ min. to dissolve pectic acid ppt. (Drops of liquid clinging to lip of bottle may be washed into 2nd bottle with small squirt of $H_2O$ from wash bottle; otherwise do not rinse at this point.) Filter, with suction, thru hardened filter (Whatman No. 41H or 54) on 11 cm Büchner funnel. Allow bottle to drain well, then rinse twice with 25 ml portions of $H_2O$ each contg. 1–2 drops of $NH_4OH$ (1 plus 1), pouring rinsings on filter, and waiting for each rinse to drain thru filter before adding another.

Decant filtrate into 250 ml centrifuge bottle, allow flask to drain, and rinse twice with 5 ml of $H_2O$. (The use of a bell jar will permit filtration direct into centrifuge bottle.) Add 5 ml of HCl to contents of centrifuge bottle, stir in 90–100 ml of alcohol, rinse off rod with alcohol (do not add filter-aid), stopper, shake, and centrifuge 8 min. at 1500–1800 r.p.m. Decant supernatant liquid into beaker, retaining most of ppt. in bottle, filter liquid thru 15 cm Whatman No. 41H filter, on a fluted funnel. Pour precipitate and liquor remaining in centrifuge tube onto filter paper and drain thoroly. (Use no rinse.) Quantitatively transfer ppt in bottle and on filter to 250 ml beaker, using total of 75 ml of 60–75° $H_2O$. Cool beaker and contents in bath (15–17°) and add, with stirring, 15% NaOH soln (also cooled) until mixt. is alk. to phenolphthalein (internal indicator). Add 3 ml in excess and allow mixt. to stand 15 min. in bath at 15–17°. During this time, heat two wash bottles, contg. respectively, the following wash solns on steam bath: "A," mixt. of 200 ml of $H_2O$, 50 ml of 95% alcohol, and 20 ml of HCl (1 plus 2.5); and "B," 400 ml of alcohol dild. to 950 ml with $H_2O$.

Remove beaker from bath; acidify contents with 10 ml of HCl (1 plus 2.5), while stirring, and dil. to 100 ml with $H_2O$. (Estimate vol. by comparison with 100 ml in similar beaker.) Add a few glass beads, cover beaker, bring contents to boil, and boil 5 min. Remove from heat, add with stirring 10 ml of HCl, and then add 400 mg of asbestos (previously alkali- and acid-washed and ignited, and free from coarse particles). Stir 40 sec., and immediately filter thru Whatman No. 41H filter on 7–11 cm Büchner funnel, using very gentle suction. (Suction should be so gentle that it can hardly be felt when thumb is placed on rubber tube before attaching tube to

flask; filtration of sample should be in small steady stream, and filtrate should be clear or only slightly opalescent, with no immediate sepn. of ppt.) Wash beaker and filter 3 times each with about 25 ml of wash soln "A," and then 4–5 times each with about 25 ml of wash soln "B" to remove acid. (Washings should be clear, and pass thru filter readily. Any appearance of precipitate in flask at this stage should be ignored.)

Place filter and ppt. on fairly large, short-stemmed funnel, and wash pectic acid ppt and asbestos into Pt dish with hot $H_2O$. Run blank on 400 mg of asbestos by adding it to hot acid soln, filtering, and drying in same manner as sample. Heat dishes on steam bath until asbestos and ppt. appear thoroly dry. Dry sample and blank in oven at 100° to constant weight (±0.2 mg: takes about one hour), cool in desiccator, weigh, ignite, cool, and weigh again. Loss in wt. of sample minus loss in wt. of blank = wt. pectic acid in aliquot taken.

$$\text{This wt.} \times \left(\frac{250}{\text{vol. of ext. taken}}\right) = \text{wt. of pectic acid in sample.}$$

To obtain dry fat-free cacao in sample, add 100 ml of 95% alcohol to cacao residue reserved in centrifuge bottle, stopper bottle, shake well, centrifuge, and decant alcohol. Again shake with 100 ml of 95% alcohol, rinse, stopper, wash down sides of bottle with 95% alcohol, using wash bottle; centrifuge, and decant. Repeat extn. but using 100 ml of ether, washing down sides, centrifuging and decanting, and allow residual ether to evaporate. By means of brush and spatula, quantitatively transfer residue to tared Al dish with cover; dry dish and contents 1–2 hrs. in oven at 100°; cover dish, cool in desiccator, and weigh. Wt. of residue × 1.9 = wt. of dry fat-free cacao in sample.

$$\frac{\text{Wt. of pectic acid}}{\text{Wt. of dry fat-free cacao}} \times 100 = \% \text{ pectic acid.}$$

(b) *In chocolate liquor, breakfast cocoa, cocoa, and low-fat cocoa.*—Place in centrifuge bottle about 15 g of cocoa or 25 g of chocolate liquor, prepd. as directed in **12.1.** To remove most of fat, shake contents of bottle thoroly with 100 ml of petr. ether (B. P. 30 to 65°), or ether; centrifuge, decant supernatant liquid, and repeat extn. with another 100 ml of petr. ether, or ether. Shake residue with 3rd portion of solvent, and filter thru Whatman No. 41H, or 54 filter on 11 cm Büchner funnel, using gentle or moderate suction. (Vacuum should be applied, and filter wetted with solvent before starting filtration.) Allow residue to suck dry, transfer it to porcelain dish or casserole, grind gently with pestle to pulverize and mix it, and transfer to Al dish with cover. Dry about 45 min. in oven at 100°, cover dish, and cool in desiccator. Weigh 5 g of the dry fat-free residue into 250 ml centrifuge bottle, and proceed as directed in (a) (1), last part of sentence, beginning "then ext. with 100 ml of 95% alcohol," and continue as directed in (a) (2), and in (a) (3) thru next-to-last paragraph (directions for calcg. wt. of pectic acid in sample).

$$\left(\frac{\text{Wt. of pectic acid found}}{5}\right) \times 100 = \% \text{ pectic acid in dry fat-free cacao.}$$

(No estimation of dry fat-free cacao is necessary, since weighed amount of dry, fat-free cacao is used for the pectic acid detn.)

## 13. CEREAL FOODS

(1) The following method for the determination of lactose in bread was adopted, first action:

### REAGENTS

*Yeast suspension.*—Wash 25 g fresh commercial baker's yeast 5 times with 4 times its volume of distilled water (100 ml) or until last washings are clear. Centrifuge and decant after each wash. Make a 25% suspension of the washed yeast and store at 0°–4°C. for 24 hours before use. (Preparations as old as one week were still satisfactory.)

*Yeast nutrient soln.*—Add 1.7 g bacto-peptone (Difco Laboratories), 0.50 g dipotassium phosphate, and 0.33 g magnesium sulfate heptahydrate to 100 ml volumetric flask, dissolve in water, make to volume.

*Protein precipitant.*—Dissolve 50 g sodium tungstate and 6 g disodium phosphate in 200 ml distilled water. Add slowly, 220 ml of 2 *N* hydrochloric acid mix, and dilute to 500 ml.

*Somogyi's reagent.*—Dissolve 12 g Rochelle salt, 20 g anhydrous sodium carbonate, and 25 g sodium bicarbonate in ca 500 ml distilled water. To this, add with stirring 6.5 g copper sulfate pentahydrate previously dissolved in 100 ml water. In a separate container, dissolve 10 g potassium iodide 0.800 g potassium iodate, and 18 g potassium oxalate in ca 200 ml water. Mix the two solns and dilute to 1 liter (if after standing a few days any small amount of deposit should be filtered off).

*Sodium thiosulfate 0.005 N.*—Prepare every day by diluting freshly standardized 0.1 *N* soln.

*Sodium hydroxide*—0.5 *N*.
*Sulfuric acid*—2.0 *N*.
*Starch soln*—1%.

### PROCEDURE

Weigh 10 g air dried bread crumb, add 5 g filtercel, mix well, transfer to extraction thimble (ca 30×77 mm), cover with cotton pad, place in Soxhlet extractor, add 150 ml alcohol-water mixture (126 ml 95% alcohol+61 ml $H_2O$) and extract overnight on hot plate set at medium heat. Transfer extract to 250 ml beaker (previously marked at 40 ml) evaporate on steam bath with aid of weak air blast to ca 40 ml, transfer to 100 ml volumetric flask, rinse well, cool, make to volume and mix well. Pipet 10 ml into a 50 ml Erlenmeyer flask, add 6 ml yeast suspension and 5 ml yeast nutrient soln. Make a blank test using 10 ml of water in place of the bread extract. Stopper the flask with a one hole rubber stopper fitted with a piece of 6 mm[1] glass tubing ca 10 cm long. Shake at a moderate rate for 2.5 hrs. in a constant temp. water bath at 30°C. Transfer to a 50 ml centrifuge tube, centrifuge for ca 10 min. (ca 1000 r.p.m.). Decant the supernatant liquid into a 50 ml volumetric flask. Rinse the Erlenmeyer flask with 10 ml $H_2O$, decanting onto the residue in the centrifuge tube. Mix the residue and water with glass rod. Centrifuge and combine washing with previous supernatant liquid in 50 ml volumetric flask. Repeat washing using 10 ml water. Add, with shaking, 2.5 ml protein precipitant. Make to volume, mix well and filter, discarding the first few ml of filtrate. (This is a convenient stopping point to stopper the flask for continuation the next day.)

Pipet 5.0 ml of the clear filtrate into a Pyrex tube (22×175 mm) neutralize to phenol red end point with 0.5 *N* NaOH. Add 5.0 ml Somogyi's reagent, mix by rotary motion, add 2 drops of benzene. Cap the tube with a glass bulb. Place in a metal rack so constructed as to prevent agitation of the tubes while in the boiling water bath. Immerse the rack containing the tubes in the vigorously boiling water bath for exactly 15 min. Cool, avoiding agitation, to ca 35°C. in a water bath. Add 2.5 ml of 2 *N* $H_2SO_4$, shake with rotary motion, allow to stand ca one or two

[1] The size of this tubing should not be smaller since occasionally loss of fermenting medium occurs thru the glass tubing during the fermentation.

min., titrate[2] the excess iodine with 0.005 *N* sodium thiosulfate, adding 6 drops of starch indicator near end of the titration. (Titrations should be finished in 30 min.) From the difference between the titration value of the blank (water, yeast suspension, and yeast nutrient) and that of the sample, determine the quantity of lactose present from the standard curve. This value in mg of lactose represents the amount in 100 mg of air dry bread or per cent lactose.

Prepare a standard curve using from 0 to 4.5 mg pure lactose hydrate (0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 4.5 mg from portions of soln containing 1.0 mg per ml in sufficient water to make 5 ml, 5.0 ml of Somogyi's reagent mixed by rotary motion, and 2 drops of benzene. Proceed as above from "Cap the tube . . . ." Plot the difference between the titration value for the zero lactose and that of the lactose soln against the corresponding lactose in mg.

(2) The first action method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol, **13.34** (p. 202), was deleted and replaced by the following method which was adopted, first action:

Weigh 20 g sample (20 mesh or finer) into a 250 ml centrifuge bottle. Add 100 ml $H_2O$ from pipet, shaking bottle to prevent lumping of sample. Add 100 ml more $H_2O$ from pipet. Shake contents of stoppered bottle one hour in shaking machine or by hand. (Preferably a horizontal shaker with bottle lengthwise. If a vertical wrist type motion machine is used, shake by hand 5 min. after the 1 hour shaking.) Temp. of $H_2O$ should not exceed 30°C. Centrifuge at 1200 r.p.m. for ca 15 min. and filter into 500 ml suction flask thru pad of asbestos (fine) on Büchner funnel (ca 2 in. diameter) using suction. Determine *N* in 50 ml of filtrate as directed under **2.22**, **2.23**, **2.24**, with a glass bead in each flask, and distilling the $NH_3$ into 20 ml of 0.1 *N* acid. Digest 1 hr. after clear. Correct for blank on reagents used in digestion.

Pipet 100 ml of above filtrate into 200 ml volumetric flask, add 15 ml NaCl soln (28 g diluted to 300 ml), fill nearly to mark with alcohol, mix well, cool to room temp., make to mark, mix and allow to stand overnight. Pipet off supernatant liquid and filter thru 18½ cm fluted filter paper (S & S 588 or equivalent). Determine *N* in 100 ml of filtrate as above, using a glass bead to avoid bumping. Add the $H_2SO_4$, mix and carefully boil off the alcohol before adding the $Na_2SO_4-HgO$ mixture. Rinse $Na_2SO_4-HgO$ mixture down neck of flask. Digest 1 hr. after clear. (Watch for foaming before clearing and keep contents out of neck of flask.) Distill into 20 ml of 0.1 *N* acid as before. Correct for blank or reagent used in digestion. Subtract this number of ml of acid used from the number of ml of acid used for water-soluble *N* and convert to per cent of water-soluble *N* precipitable by 40% alcohol.

(3) The following first action methods for baked products other than bread (not containing fruit) (p. 212) were adopted as official:

**13.86.** Solids.
**13.87.** Ash.
**13.88.** Protein
**13.89.** Fat.
**13.90.** Crude Fiber.

(4) The first action method for the determination of acetic and propionic acids in bread, *This Journal*, **34**, 64–68 (1951), was adopted as official.

[2] For stirring the soln during titration, a rod made of glass tubing, sealed and flared at the lower end to form a button-like foot, is convenient. A side arm consisting of several layers of adhesive tape may be made near enough the top of the glass tubing to prevent breaking the bottom of the titration tube.

(5) The first action Method I for nitrite nitrogen, *This Journal*, **34**, 68 (1951), was adopted as official.

(6) The first action method for carotene in noodles, *This Journal*, **34**, 68–70 (1951), was adopted as official.

### 14. COFFEE AND TEA

No additions, deletions, or other changes.

### 15. DAIRY PRODUCTS

(1) The following hand-stirring method for sampling commercial containers of cottage cheeses was adopted as a procedure:

Stir the can thoroly for at least 5 min. with a dairy stirrer (ca 5½ inch perforated concave metal disk attached to ca 27 inch metal rod as a handle) so that all portions of the container are reached. Remove portions from the top with a small spoon to fill a pint jar, and cover.

(2) The official method for fat in ice cream and frozen desserts, **15.153** (p. 273), was modified as follows and adopted as official:

Substitute for the first sentence the following: "Weigh accurately 4–5 g of thoroly mixed sample directly into a Mojonnier flask (or Röhrig tube or similar apparatus), using a short, bent, free flowing pipet; dilute with $H_2O$ to ca 10 ml, working the charge into a lower chamber and mixing by shaking.

(3) (a) Par. **15.104(c)** (p. 256), *Sample containers* was changed by deletion of "preferably with glass tops," and adopted as official; (b) paragraph **15.105** (p. 256) was editorially revised as follows, and adopted as official:

Soften entire sample in the sample container **15.104(c)** by warming in a water bath maintained at as low a temp. as practicable, not over 39°. Avoid overheating which results in visible separation of curd. Shake at frequent intervals during the softening process to reincorporate any separated fat and observe fluidity of sample. Optimum consistency is attained when the emulsion is still intact but fluid enough to reveal almost immediately the sample level. Remove from bath and shake vigorously at frequent intervals until sample cools to a thick creamy consistency and sample level can no longer readily be seen. Weigh portion for analysis promptly.

(4) The mechanical stirrer Method I for the preparation of butter samples, **15.106–15.107** (p. 256), was deleted.

(5) The following methods were adopted as official:

**15.19–15.20** (p. 232), Casein, Method II.

**15.95** (p. 251), Casein in malted milk and chocolate malted milk.

**15.98** (p. 253), Fat in malted milk.

**15.47** (p. 242), Residual phosphatase, Method II, for products other than fluid milk: **15.122** (p. 262), butter; **15.144–15.147** (p. 268), other cheeses; **15.157** (p. 273), ice cream and frozen desserts.

(6) The five methods listed in *Changes in Methods of Analysis*, Chapter 22, Dairy Products (6), *This Journal*, **33**, 80 (1950), as being made first action, were actually adopted as official.

(7) The deletions provided in item (3) for **15.28,** *This Journal,* **34,** 70 (1951), should read as follows: "Paragraph **(a)** delete 'Reading below 39 indicates added $H_2O$; between 39 and 40, addition of $H_2O$ is suspected.' and 'When reading is 40 or below, det. ash in serum as directed under **(b).**' " "Paragraph **(b)** delete 'Result below 0.715 g/100 indicates added $H_2O$.' "

(8) The deletions provided in item (4) for **15.29,** *This Journal,* **34,** 70 (1951), should read as follows: " 'Reading below 36 indicates added $H_2O$.' and 'When refractometer reading is 36 or below, det. acetic serum ash as directed under **15.28(b).**' "

### 16. EGGS AND EGG PRODUCTS

(1) The first action method for succinic acid, **16.34–16.39** (p. 284), was adopted as official.

### 17. ENZYMES

No additions, deletions, or other changes.

### 18. FISH AND OTHER MARINE PRODUCTS

No additions, deletions, or other changes.

### 19. FLAVORING EXTRACTS

(1) The following changes were made in the photometric method for vanillin in imitation vanilla flavors, *This Journal,* **34,** 72 (1951), and the revised method was adopted, first action.

(a) Under Preparation of Graph (p. 73), line 4, delete "18.5 cm."

(b) Under Determination of Sample (p. 73) add the following paragraph:

NOTE: Use No. 12 Whatman 18.5 cm folded filter paper or equivalent on all filtrations. When the concentration of vanillin exceeds 1.0 g per 100 ml of sample, dilute 50 ml of sample to 100 ml with water and use diluted sample. When determination is made with a photoelectric spectrophotometer, dilute samples and standards to double the volume directed above and read at a wave length of 610 mμ.

(2) The following changes were made in the photometric method for coumarin in imitation vanilla flavors, *This Journal,* **34,** 73 (1951), and the revised method was adopted, first action:

(a) Under Preparation of Graph (p. 74), line 5 from top of page, delete "11 cm S & S 589" and in line 10 delete "S & S 589."

(b) Under Determination of Sample (p. 74), add the following paragraph:

NOTE: Use No. 12 Whatman 18.5 cm folded filter paper or equivalent for all filtrations. When determination is made with a photoelectric spectrophotometer, dilute samples and standards directed above to double volume and read at a wave length of 490 mμ.

(3) The following method for isopropyl alcohol (applicable in presence of acetone) in lemon and orange flavors was adopted, first action:

APPARATUS

*Model DU Beckman Spectrophotometer*, or its equivalent, and matched 1 cm quartz cells.

*Glassware.*—Use foil-wrapped stoppers or preferably an all glass still. Provide the condenser with an adapter which reaches several inches into the volumetric flask.

DETERMINATION

Proceed by **19.23**, placing the 100 ml volumetric flask in an ice and water bath.

*Acetone.*—Pipet a suitable aliquot, preferably containing 0.1–0.3 g acetone, into a 100 ml volumetric flask, make to the mark and determine the optical density at 265 mμ with distilled water as the reference soln. Make readings of water in the same cell as is used for the sample soln and deduct this blank from the E-value obtained above. The quantity of acetone present in the 100 ml flask is

$$C = \frac{E_{1\text{ cm}}}{3.08} \qquad \text{where} \quad C = \text{grams acetone/100 ml}$$
$$\text{and} \quad 3.08 = E_{1\text{ cm}}^{1\%} \text{ for acetone}$$

Calculate the acetone to g/100 ml.

*Isopropyl Alcohol.*—Into a 500 ml flask containing 50 ml of ca 2 *N* $K_2Cr_2O_7$, pipet 10 ml of the distillate from **19.23** and add 100 ml of $H_2SO_4$(1 +3). Stopper the flask, swirl, and let stand 30 min. Add 100 ml of 30% $FeSO_4 \cdot 7\ H_2O$ soln. Connect flask to a vertical condenser thru a foam trap. Slowly distill about 100 ml into a 250 ml volumetric flask containing about 100 ml of cold water, and held in an ice and water bath. Dilute to mark and determine the optical density as directed above. The quantity of acetone present in the 250 ml flask is:

$$C = \frac{2.5\ E_{1\text{ cm}}}{3.08}\ \text{g/250 ml.}$$

Calculate to g/100 ml of original sample. Deduct the free acetone and multiply by 1.0347 to give the quanity of isopropyl alcohol.

(4) The following method for essential oil in emulsions was adopted, first action:

APPARATUS

*Boiling flask.*—Use a 500 ml round bottom flask with ground glass joint 24/30.

*Oil trap.*—Use the modified oil separatory trap described in *This Journal*, 27, 201 (1944).

*Condenser.*—Use a tightly fitting finger condenser having a projection at the bottom to facilitate return of the oil to the oil trap.

DETERMINATION

Place ca 200 ml of $H_2O$ in boiling flask; measure 5–10 ml of the emulsion (believed to contain not over 2 ml of essential oil) in a tared glass-stoppered graduated cylinder and find the weight of the oil; add to the flask and wash out the cylinder into the flask by adding several 5 ml portions of $H_2O$ to the cylinder and shaking to remove the emulsion from the sides.

Fill the oil trap with $H_2O$ until it overflows, connect with the boiling flask and condenser and carefully boil for one hour. Remove the heat source and let stand for several min. Draw off enough water to bring the oily layer within the graduated portion of the trap, let stand for 5 min. to complete drainage, and measure the quantity of oil from bottommost to topmost points of the menisci.

(5) The following method for essential oil in citrus juices and other beverages was adopted, first action:

APPARATUS

*Boiling flask.*—Use a 2 liter flask with ground glass joint 24/40.

*Oil trap.*—Use the modified oil separatory trap described in *This Journal*, **27**, 201 (1944).

*Condenser.*—Use a tightly fitting finger condenser with a projection on the bottom to facilitate return of the distillate to the oil separatory trap.

DETERMINATION

Place 1 liter of sample in the boiling flask and add a few glass beads to facilitate boiling. Fill the oil separatory trap with $H_2O$, connect with the boiling flask and condenser and boil for one hour. Remove the heat source and let stand several min. Draw off enough water to bring the oily layer within the graduated portion of the trap, let stand at least 5 min. to complete drainage, and measure the quantity of oil from bottommost to topmost points of the menisci.

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

## 20. FRUITS AND FRUIT PRODUCTS

(1) The first action method for citric acid, *This Journal*, **34**, 74–75 (1951), was adopted as official, with the following corrections: in paragraph headed "Removal of Pectin," line 3, insert "*N*" between "ml" and "$HNO_3$." Last paragraph, last line, change "64" to "0.64."

## 21. GELATINE, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or other changes.

## 22. GRAINS AND STOCK FEEDS

(1) The first action method for sulfaguanidine, *This Journal*, **34**, 75 (1951), was adopted as official.

(2) The following method for cobalt in mineral feeds was adopted, first action:

REAGENTS

*Cobalt sulphate* ($CoSO_4 \cdot 7H_2O$).—Do not dry, use as received. Dissolve 0.2385 g in water and make up to 1 liter. 1 ml = 0.05 mg Co. This soln may be diluted to suitable concentration in making standard curve.

*Nitroso-R-Salt* ($C_{10}H_4OH \cdot NO(SO_3Na)_2$).—Dissolve 1 g in water and make up to volume of 500 ml.

*Spekker acid.*—Mix 150 ml 85% $H_3PO_4$ and 150 ml $H_2SO_4$ and make up to volume of 1 liter with water.

*Sodium acetate* ($NaC_2H_3O_2 \cdot 3H_2O$).—Dissolve 500 g in water and make up to volume of 1 liter.

STANDARDS

To 1, 2, etc., up to 11 ml of standard cobalt sulfate solutions in 100 ml volumetric flasks are added 2 ml of Spekker acid, 10 ml of Nitroso-R-Salt soln, and 10 ml of sodium acetate soln. A blank is made by using 2 ml of Spekker acid and 10 ml of sodium acetate soln, but *omitting* the Nitroso-R-Salt. The blank and standard solns are brought to a boil on the hot plate. Five ml of $HNO_3$ are added and the solns are boiled for at least 1 min. and not more than 2 min. The standards and blank are then cooled and diluted to 100 ml with water.

PROCEDURE

Ash 2 g of sample 2 hrs. at 600°C., transfer to 200 ml volumetric flasks with 20 ml of HCl and 50 ml of $H_2O$, boil 5 min., make to volume. Allow soln to settle. Pipette a suitable aliquot into a small flask. For samples containing 0.01–0.2% cobalt use 0.25 g sample. Use more or less according to cobalt expected. The maximum quantity of cobalt permissible in a sample is 0.5 mg, as above this concentration the soln no longer appears to follow Beer's law. Pass a brisk current of hydrogen sulfide thru the soln for 10 min. Filter directly into a 100 ml Pyrex volumetric flask. Use a No. 40 Whatman filter paper. Wash with ca 50 ml of acidulated $H_2S$ water (1% $H_2SO_4$). Add 2 small glass beads to flask and boil off $H_2S$. (Flasks must be given individual attention as violent bumping may occur.) Supplement glass beads by often shaking flask. Add 5 ml $HNO_3$ and boil until nitrous fumes no longer appear. (Care must be taken at this point, as the soln will be low and spattering and bumping may occur.) At first indication of bumping or spattering, remove immediately from hot plate. The small amount of nitric acid remaining will not affect the result. Cool. Add 2 drops of phenolphthalein and take to first faint pink with NaOH (ca 30% soln). Immediately add 2 ml of Spekker acid followed by 10 ml of nitroso-R-salt soln and 10 ml sodium acetate soln. Bring to vigorous boil, carefully add 5 ml $HNO_3$ and then boil at least one min. but not over 2 min. Cool and make to volume.

Compare color with standard cobalt solns in colorimeter, using a green or No. 54 filter. If using a spectrophotometer, a wave length of 540 millimicrons is recommended. Reading of color should be made within 2 hours. Report percentage of cobalt to the third place to right of decimal (.000%).

## 23. MEATS AND MEAT PRODUCTS

The following changes were made in the first action method for nitrites, **23.15–23.16** (p. 361), and the revised method was adopted, first action:

(a) Paragraph **23.15** (p. 361–2). Delete reagents **(a)** and **(b)** and substitute the following:

**(a)** *Modified Griess reagent.*—Dissolve 0.5 g sulfanilic acid in 150 ml 15% acetic acid. Boil 0.1 g alpha-naphthylamine in 20 ml $H_2O$ until dissolved and pour while hot into 150 ml 15% acetic acid. Mix the 2 solns and store in brown glass bottle.

(b) Paragraph **23.15** (p. 362). Change reagent **(c)** to **(b)**.

(c) Paragraph **23.16** (p. 362). Delete, starting with the sentence in line 8 "Filter, dil. suitable aliquot to mark . . . " to the end of the paragraph, and substitute the following:

Filter, dilute suitable aliquot to mark in 50 ml vol. flask, add 2 ml reagent, mix, and allow color to develop for 1 hr. Transfer suitable portion of soln to photometer cuvette and determine density at a wave length of 520 millimicrons, setting the instrument to zero density by means of a blank consisting of 50 ml $H_2O$ plus 2 ml reagent. Determine nitrite present by comparison with standard curve prepared as follows: Dilute suitable volumes of standard nitrite soln to mark in 50 ml vol. flasks, add 2 ml of reagent, and proceed as above.

## 24. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

(1) The following method for mercury was adopted, first action:

PRINCIPLES

The procedure consists of wet digestion of the sample with nitric and sulfuric

acids under reflux in a special apparatus; isolation of mercury by dithizone extraction; removal of copper; estimation of mercury by photometric measurement of mercuric dithizonate.

### PRECAUTIONS

The critical part of procedure is digestion of sample. Unless sample digestion is almost complete, residual organic matter may combine with mercury and prevent or hinder extraction with dithizone. Oxidizing material in digest must also have been destroyed or dithizone reagent is decomposed and mercury is not quantitatively extracted. Because of volatility of mercury compounds, careful heating of digest during sample preparation is required. The acidity of final sample solution (after partial neutralization with ammonium hydroxide) prior to extraction should be ca 1 *N* and no higher than 1.2 *N*.

### APPARATUS

(1) Digestion apparatus—see Figure 1.

(a) A 500 ml two neck standard taper round bottom flask.

(b) Standard taper modified Soxhlet unit devoid of syphon overflow, but equipped with stopcock on tube leading to digestion flask.

(c) Standard taper condenser affixed to top of Soxhlet unit. When stopcock of latter is open, assembly is in reflux position. When closed, unit serves as trap for condensed water and acids.

(d) Standard taper dropping funnel connected to outer vent of digestion flask.

Unit A, modified Soxhlet, 5 cm O.D., 200 ml capacity before overflow. Unit B, dropping funnel, ₮ 24/40, capacity 75 ml. C, Friedrichs condenser, 35 cm total length. D, 500 ml digestion flask with two ₮ 24/40 holes, 3 cm apart, for clearance of A and B.

(2) Separators (preferably Pyrex) of 250 and 500 ml or liter volume.

NOTE: As mercury compounds tend to adsorb on glassware, the apparatus, particularly separators, should be rinsed with dilute nitric acid and then distilled water before an analysis.

### REAGENTS

All reagents must be of analytical quality.

(1) *Mercury standard.*—Prepare from dry, recrystallized mercuric chloride. A convenient stock soln is 1 mg mercury per ml. Prepare dilute standard solns from stock soln and store in Pyrex glass. Add conc. hydrochloric acid in the proportion of 8 ml per liter to all standards before diluting to final volume. A convenient dilute standard is 2 mmg per ml.

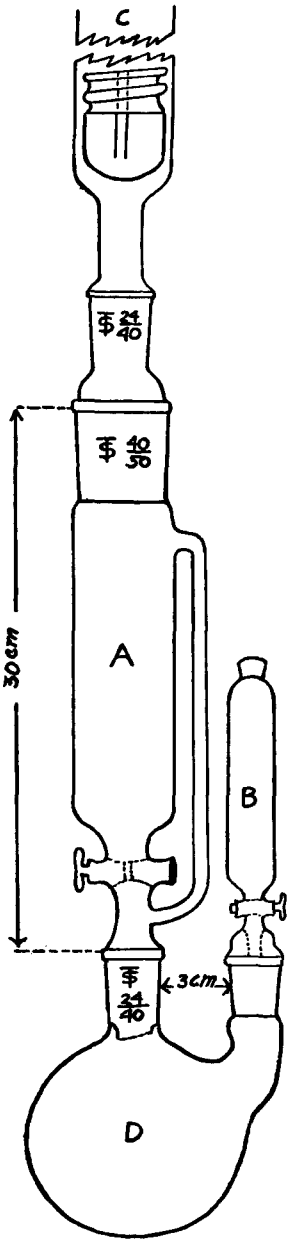


FIG. 1.—Special Digestion Apparatus

(2) *Chloroform.*—Distil from hot water bath and collect distillate in brown bottle containing 10 ml absolute ethyl alcohol per liter distillate. Swirl receiver intermittently during the distillation.

(3) *Dithizone.*—The reagent as now distributed needs no further purification. A convenient stock soln is 100 mg/liter in reagent (2). Store in refrigerator when not using. As dilute solns of dithizone are somewhat unstable, prepare dilutions from the stock as needed.

(4) *Sodium thiosulfate 1.5% w/v.*—Prepare daily.

(5) *Sodium hypochlorite.*—C. P. Preferably 5% available chlorine reagent. As distributed, reagent varies in available chlorine content. Determine strength by analysis. Store in refrigerator when not using and determine titer at monthly intervals. (Certain preparations of hypochlorite intended for household use contain traces of mercury. If these preparations are used, run careful blank. A reagent with more than 0.1 mmg Hg per ml should not be used.)

(6) *Acetic acid,* 30% by volume.

(7) *Hydroxylamine hydrochloride 20% w/v.*—Extract with dilute dithizone until chloroform layer remains green, remove excess dithizone with chloroform, and filter

### SAMPLE PREPARATION

*(Conduct Acid Digestion in Hood)*

In all determinations use amount of sample equivalent to no more than 10 g, dry weight.

(a) *Fresh fruits or vegetables and beverages.*

Place weighed sample into digestion flask along with 6 glass beads, connect assembly, and add thru dropping funnel 20 ml of conc. nitric acid. Send rapid stream of water thru condenser, adjust stopcock of Soxhlet unit to reflux position, and apply small flame to flask. Use an asbestos board with 1–2″ diameter hole between flask and flame. (The original reaction must not proceed violently or the evolved nitrogen dioxide will carry vapors of the digest mechanically thru condenser and cause loss of mercury.) After initial reaction is complete, apply heat so that digest just refluxes. Should the mixture darken, add nitric acid dropwise thru funnel as needed. Continue refluxing for 0.5 hr, or until digest does not change consistency. Allow to cool, add slowly 20[1] ml of cold nitric-sulfuric acids (1+1). Heat with small flame, adding subsequently nitric acid dropwise as needed to dispel darkening of the digest. Continue heating until fibrous material (fruit skin, cellulose, etc.) is apparently digested. Turn stopcock of Soxhlet unit to trap water and acids and continue heating. Allow digest to become dark brown (not black) before adding further increments of nitric acid. (Fats and waxes can not be totally digested by the hot acids under reflux. No attempt should therefore be made to effect a complete digestion in this step.) When all but fat and wax is in solution, allow digest to cool and drain water and acids cautiously into the main digest. Cool and pour two 25 ml portions of water thru condenser and intermediate unit. Remove reaction flask, chill under cold water or by surrounding with ice to solidify fats and waxes, and filter off insoluble matter on a small pledget of glass wool. Rinse reaction flask and filter pad successively with two 10 ml portions of water. Remove Soxhlet unit and wash it and the flask with hot water to remove insoluble material. Pour hot water thru condenser to remove volatile fats and oils. Discard all washings. Connect flask containing filtered sample soln to assembled apparatus, heat, and collect water and acids in the trap. Complete the digestion, using small additions of nitric acid as needed.

[1]Use 10 ml acid mixture for 5 g or less (dry weight) of sample.

In final stage of digestion, adjust flame until digest reaches incipient boiling (soln simmers) and acid vapors do not rise beyond lower half of condenser. Continue heating for 15 min. after last addition of nitric acid. Digest should now be colorless or pale yellow. Allow digest to cool, drain trapped liquids carefully into reaction flask, and add two 50 ml portions of water thru condenser. Reflux soln until all nitrogen dioxide is dispelled from apparatus. Add 5 ml urea reagent 40% w/v and reflux 15 min. (Digest should be colorless or a pale yellow soln.)

**(b)** *For dried fruit, cereal, seeds, and grains.*

Dilute sample with 50 ml of water before adding nitric acid, and proceed with sample preparation as described in **(a)**.

**(c)** *For meats, fish and biological material.*

Because of high fat and protein content of these materials, initial digestion must be conducted carefully to avoid foaming of digest into condenser. Add 20 ml of nitric acid to sample, swirl flask, and let stand 0.5 hr. in digestion assembly before heating. Add 25 ml of water and heat cautiously with small rotating flame until initial vigorous reaction is over and foaming has ceased. Proceed therefrom as described in **(a)**.

Titrate 1 ml of sample soln thus prepared with standard alkali. Add the calculated amount of concentrated ammonium hydroxide to reduce the acidity to 1.0 *N;* swirl the flask during the addition of the ammonia in order to avoid local excess. (Soln should never be ammoniacal to avoid formation of mercury complexes.)

ISOLATION OF MERCURY

The following table is useful in preparing standard curve and for establishing approximate mercury range in sample solution.

| *Mercury Range mmg* | *Dithizone Conc. mg/l* | *Volume Dithizone ml* | *Cell Length cm* |
|---|---|---|---|
| 0–5 | 4 | 10 | 5 |
| 0–10 | 5.5 | 10 | 2.5 |
| 0–50 | 10 | 25 | 2.5 |
| 0–100 | 15 | 25 | 1 |

Transfer sample soln to 500 ml separator. Add 10 ml of 4 mg/l dithizone and shake vigorously for 1 min. (If green color of dithizone is apparent in chloroform layer, indicating excess of dithizone, the amount of mercury is within 0–5 mmg.) Allow layers to separate and transfer chloroform layer quickly to a second separator containing 25 ml of 0.1 *N* HCl and 5 ml of reagent **(7)**. (A small amount of oxidizing material may still be present. On long contact with dithizone soln, oxidizing substances may destroy dithizone reagent and prevent extraction of mercury.) Repeat extraction of sample soln with two additional 5 ml of dithizone, transferring chloroform layer successively to the second separator. If the first extraction indicates mercury in excess of 5 mmg add stronger concentrations of dithizone, as indicated by the table, until, after 1 min. vigorous shaking, the chloroform layer contains dithizone in marked excess. Drain chloroform layer into second separator containing the 0.1 *N* HCl and repeat extraction of the sample soln with two 10 ml portions of 4 mg Dz/l concentration of dithizone, draining each successive extract into the second separator. Shake contents of second separator vigorously for 1 min., and drain chloroform layer into third funnel containing 50 ml of 0.1 *N* HCl. (Shaking dithizone extract with dilute acid in second funnel removes entrained organic matter which may be present. With biological materials or those of high protein content,

aqueous layer is usually light yellow because of nitrated organic compounds. Small amounts are carried into third funnel where they are destroyed by chlorine.) Extract soln in second funnel with 1–2 ml chloroform and transfer organic layer to third separator. To contents of third separator add 2 ml thiosulfate reagent, shake vigorously for 1 min., allow layers to separate, drain off chloroform as completely as possible and discard. (Copper if present is thus removed as the dithizonate.) Extract again with 1–2 ml of chloroform, drain carefully, and discard. Add 3.5 ml of hypochlorite reagent (or amount of soln of different titer sufficient to furnish 175 mg of available chlorine) to decompose mercury thiosulfate complex and to oxidize excess thiosulfate, and shake vigorously 1 min. Add 5 ml of hydroxylamine hydrochloride reagent (7) from a pipette, taking care to wet both stopper and neck of separator and shake vigorously 1 min. Hold mouth of separator in front of air vent and blow out any remaining gaseous chlorine. Stopper funnel and shake again vigorously for 1 min. (It is imperative that all hypochlorite be reduced. Traces of the reagent remaining would oxidize dithizone, subsequently added, to yellow oxidized form which would be measured in the photometer as mercury.) Extract soln with 2–3 ml of chloroform, drain off organic layer carefully, and discard. The final aqueous soln should now be colorless. Proceed as directed under Determination.

#### PREPARATION OF STANDARD CURVE

Prepare working curve of required range, starting with blank and extending to final standard of range, with four intermediate increasing increments. Add appropriate amounts of mercury to 50 ml of 0.1 *N* HCl in separator. Add 5 ml of hydroxylamine hydrochloride reagent, and 5 ml of chloroform, and shake vigorously 1 min. Allow layers to separate, drain off chloroform and discard, being careful to remove as completely as possible all droplets of chloroform. Add 3 ml of acetic acid reagent, the appropriate volume of dithizone soln, shake vigorously 1 min. and allow layers to separate. (The acetic acid aids in stabilizing mercuric dithizonate.) Insert pledget of cotton into stem of separator and collect dithizone extract (discarding first ml) in a test-tube for transfer to appropriate transmission cell. Make photometer readings at 490 m$\mu$. (Since both dilute dithizone and mercuric dithizonate are somewhat unstable, the photometric readings should be taken immediately.)

#### DETERMINATION

To contents of third separator add 3 ml of acetic acid reagent and appropriate volume and concentration of dithizone soln, as indicated by chart, and proceed with colorimetric evaluation of mercury as described under Preparation of Standard Curve, converting optical density, measured at 490 m$\mu$, to mmg Hg from the working curve.

### 25. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

### 26. OILS, FATS, AND WAXES

(1) The first action method for coal-tar colors in oil, *This Journal*, **34**, 76–78 (1951), was adopted as official.

(2) The following method for propyl gallate was adopted, first action:

#### REAGENTS

(1) *Petroleum ether reagent.*—Mix one volume of good quality 30–60°C. pe-

troleum ether (**13.67** or equivalent), with 3 volumes of 60–100°C. petroleum ether (Skellysolve B and H have been found satisfactory) and shake the mixture with one-tenth its volume of concentrated sulfuric acid for 5 min. Discard the acid layer, wash several times with water, then once with 1% NaOH soln, and then again with water until washings are substantially neutral. Discard all washings and distill the petroleum ether in an all-glass apparatus, using suitable precautions.

(2) *Ammonium acetate solns.*—1.25%, 1.67%, and 10% w/v aqueous solns. A soln containing 1.67% of ammonium acetate in 5% aqueous ethyl alcohol may also be required.

(3) *Ferrous tartrate reagent.*—Dissolve 0.100 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and 0.500 g of Rochelle salt ($NaKC_4H_4O_6 \cdot 4H_2O$) in distilled water and make up to 100 ml. The reagent should be freshly prepared (must be used within three hours of preparation).

### PREPARATION OF STANDARD CURVES

Prepare a standard aqueous soln of propyl gallate containing 50 micrograms per ml (dissolve 50 mg PG in a liter of distilled water), and place suitable aliquots, covering the range from 50 micrograms to 1000 micrograms, in 50 ml glass stoppered Erlenmeyer flasks. At least seven points should be plotted to cover the range adequately. Add exactly 2.5 ml of 10% ammonium acetate to each flask, make up to exactly 24 ml with distilled water, and then pipette into each flask 1 ml of ferrous tartrate reagent. Let solns stand at least 3 min., then measure the absorbancies at 540 mμ relative to a soln containing 20 ml of 1.25% ammonium acetate soln, 4 ml of water, and 1 ml of ferrous tartrate soln.

### DETERMINATION

Dissolve 40 g of the fat or oil in the purified petroleum ether reagent and make up to 250 ml. (Gentle warming may be necessary to obtain complete solution.) Pipette 100 ml of the fat soln into a 250 ml separatory funnel. Extract the fat soln with 20 ml of the aqueous 1.67% ammonium acetate soln by continuously inverting the separatory funnel for 2.5 min. After the phases separate completely, withdraw the aqueous layer into a 100 ml volumetric flask being careful not to allow any oil droplets to fall into the flask.[1] Repeat the extraction twice more with 20 ml portions of 1.67% ammonium acetate soln, combining the aqueous layers in the volumetric flask. Finally, extract the fat soln with 15 ml of water for 30 seconds and combine the aqueous layer with the previous washings. (Time must be allowed after each washing for the layers to separate completely.) Add exactly 2.5 ml of 10% ammonium acetate soln to the combined extracts in the volumetric flask and make to volume with water. This soln now contains 1.25% ammonium acetate. Filter thru dry rapid filter paper to remove any turbidity. (Colors must be developed on same day extract is prepared. If the combined extracts are allowed to stand for more than several hours, a yellow color may develop, and the solns must be discarded.)

Pipette aliquot of the extract, not exceeding 20 ml, into 50 ml glass stoppered Erlenmeyer flask. Dilute aliquot to 20 ml with 1.25% ammonium acetate soln. Add exactly 4 ml of distilled water and pipette 1 ml of ferrous tartrate reagent into the flask. Mix well, and measure the absorbancies at 540 mμ relative to a soln containing 20 ml of 1.25% ammonium acetate soln, 4 ml of water, and 1 ml of ferrous tartrate reagent.

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[1] In the case of some shortenings, a strong tendency to emulsify may be noted during aqueous extraction of propyl gallate. To prevent this emulsification, add 2 ml of *n*-octanol to the fat soln aliquot before beginning the extraction. A 1.67% ammonium acetate soln in 5% ethyl alcohol should be used for the extraction in place of the aqueous soln. This procedure need only be used when the normal method fails.

## 27. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The following method for the detection of propoxy-2-amino 4-nitrobenzene (P-4000) was adopted, first action:

*Organoleptic test.*—Make alkaline (*p*H 7.5–8.0) with 10% NaOH 200 ml of liquid food or aqueous extract of 200 g of solid food or semi-solid product, **27.41(c)**, and extract 3 times with 25 ml portions of petroleum ether. Wash combined petroleum ether extracts once with 5 ml of $H_2O$, transfer to small beaker or empty dish, allow ether to evaporate spontaneously, and taste residue. (Presence of as little as 5 mg of P-4000 per liter or kg of original material may be detected by its intensely sweet taste, or as little as 12.5 mg/l or kg of original material may be detected by its strong anesthetic effect.)

(2) The following method for the detection of cyclohexylsulfamate (sucaryl sodium®) was adopted as first action:

*Sodium nitrite test.*—Add to 100 ml of sample or of aq. ext., prepared as directed in **27.45(c)**, 2 g of $BaCl_2$. Allow to stand 5 min. and filter. Acidify with 10 ml HCl and add 0.2 g $NaNO_2$. A white precipitate ($BaSO_4$) indicates presence of cyclohexylsulfamate.

(3) The first action Modified La Parola-Mariani Test for dulcin, **27.10** (p. 457), revised as follows, was adopted as official:

*La Parola-Mariani Test.*—Expose dry residue, **27.8**, to HCl gas for 5 min. and add 1 drop of anisaldehyde. Presence of dulcin is indicated by development of orange-red to blood red color. (Presence of as little as 25 mg/l or kg of original sample can usually be detected by this test.)

## 28. SPICES AND OTHER CONDIMENTS

(1) That the first action method for ether extract in prepared mustard **28.31** (p. 483), modified as follows, was adopted as official:

Delete all of the last sentence beginning with "as directed under, etc." and substitute "by extracting for 16 hours with anhydrous ether in a Soxhlet extractor with Whatman Single Thickness or other close-textured thimble. Dry extract as directed under **22.25**."

(2) The following method for the preparation of samples of separable types of french dressing was adopted as a procedure:

### PREPARATION OF SAMPLE

Weigh bottle containing sample. Shake bottle for one minute and empty contents into Waring blendor. Let bottle drain for one minute. Weigh empty bottle to determine weight of sample. Add 0.20 g of egg albumin powder per 100 g of sample and place cover on blendor. Stir for 5 min.; then transfer to suitable container of larger capacity than volume of sample. Shake sample ca 20 times, and stir with spatula or spoon ca 20 times, before each portion is removed for analysis. Make all weighings immediately after preparation of sample.

Correct analytical results for added emulsifier.

(3) The subtitle "Mayonnaise and Salad Dressing" before paragraph **28.38** (p. 484), was changed to "Dressings for Foods."

(4) The first action method for total fat in dressings for food **28.46** (p. 485), was adopted as official.

(5) The following method for the detection of gums in salad dressing was adopted, first action:

REAGENTS

(a) *Calcium chloride soln.*—Density 1.2 at 20° C. If cloudy, allow soln to stand so insoluble matter may precipitate out and then filter.

(b) *Iodine soln.*—Dissolve 2 g of iodine and 6 g of KI in distilled $H_2O$ and make to 100 ml.

(c) *Ethanol.*—95%.

(d) *Ethanol.*—70%.

(e) *Basic lead acetate.*— 29.18(a).

(f) *Celite Filter Aid* or equivalent.

SEPARATION OF GUMS FROM STARCH

Defat 50 g of salad dressing by heating on steam bath in 250 ml beaker until fat separates, cool and extract with petroleum ether until last ether extract is colorless. Make alkaline with $MgCO_3$ (2–2.5 g), testing with Alkacid test paper. Heat mixture in water bath at 80°C. until residual ether and $CO_2$ are expelled. Then add 100 ml of $CaCl_2$ soln and heat in boiling water bath for 30 min. with occasional stirring. Pour into 250 ml Pyrex centrifuge bottle, centrifuge and decant as much of supernatant material as possible into 250 ml separatory funnel. Add 10 ml of $CaCl_2$ soln to residue in bottle and shake well. Centrifuge and decant supernatant material as before into separatory funnel. Swirl funnel gently and allow oil to separate. Draw off all material below oil into another 250 ml Pyrex centrifuge bottle. Centrifuge and filter supernatant liquid thru 11 cm Büchner funnel with aid of Celite filter aid. The filter paper, Whatman No. 1 or equivalent, should be precoated with layer of filter aid. Collect filtrate in beaker within bell jar. Add 10 ml of $CaCl_2$ soln to residue in centrifuge bottle, shake well, centrifuge and decant supernatant material upon filter in Büchner funnel. Wash filter with sufficient $CaCl_2$ soln so that total amount of filtrate collected is ca 110 ml.

Add slowly and with stirring 20 ml of iodine soln to clear extract and to precipitate starch-iodide and a small quantity of Celite filter aid to help filtration. (Iodine should be present in considerable excess over that amount required to quantitatively react with starch. Considerable amounts of reducing substances are present, which must be satisfied before starch can be quantitatively separated.)

Allow starch-iodide, which separates in finely divided condition to stand ca 1 hr and then filter off by suction thru 11 cm Whatman No. 1 or equivalent filter paper, precoated with adequate layer of Celite filter aid. Use wire screen under filter paper to aid filtration. Do not wash pad. (Test for excess iodine in filtrate should be made with starch iodide paper or starch soln. This test must be positive to insure removal of all starch.) To brown-colored filtrate add four volumes of 95% ethanol and let stand overnight. Centrifuge off precipitated crude gum. Wash twice with 70% ethanol. (If possible, gum should be transferred into centrifuge bottle, but in some cases gum adheres so firmly to wall of beaker that it can only be rinsed until washings are clear.)

Heat at 100°C. until alcohol is removed and dissolve residue in 20 ml of $H_2O$ by heating in water bath until no more material will dissolve. Use rubber policeman to assist solution. (Be sure gum is dissolved or it will be lost here.) Centrifuge to remove any insoluble material. Decant supernatant liquid into another 250 ml centrifuge bottle, add one drop of glacial acetic acid, one drop of $CaCl_2$ soln and reprecipitate with four volumes of 95% ethanol. Allow to stand at least one hour or overnight. Centrifuge and wash precipitate twice with 70% ethanol by shaking well and centrifuging.

Again drive off alcohol with aid of gentle stream of air by heating in hot water bath and dissolve precipitate in 10 ml of hot water, using rubber policeman. (Heed warning in preceding paragraph.) Centrifuge to remove any insoluble matter and decant into 50 ml heavy duty pyrex centrifuge tube. (Short cone type is less liable to break.) Adjust volume to 10 ml, add one drop of glacial acetic acid, one drop of $CaCl_2$ soln, and reprecipitate with 40 ml of ethanol. Allow to stand one hour, centrifuge, and wash with 70% ethanol as before. Heavy flocculent precipitate at this point indicates presence of gums. (Very small quantity of precipitate adhering to walls of centrifuge tube or appearing as mere turbidity is to be disregarded, as spice gums present in most salad dressings will usually give such precipitate.)

#### DETECTION OF GUM

To confirm presence of gums, remove residual alcohol by gentle heating in hot water bath, dissolve residue in 10 ml of hot water, and centrifuge to remove any insoluble material. Decant supernatant liquid into 10 ml graduated cylinder, make up to 10 ml with distilled water and mix. To one ml of this soln add one or two drops of basic lead acetate reagent (e), one drop at a time. An immediate flocculent, curdy or gelatinous precipitate is confirmation of presence of gums. A precipitate may form on standing but this is to be disregarded.

## 29. SUGARS AND SUGAR PRODUCTS

(1) The following Folin and Wu micro method for the determination of dextrose was adopted, first action:

#### REAGENTS

(a) *Phosmolybdic acid soln.*—Add 200 ml 10% NaOH soln and 200 ml $H_2O$ to 35 g of molybdic acid and 5 g sodium tungstate in a liter beaker. Boil vigorously 20–40 min. Cool, dil. to ca 350 ml, add 125 ml $H_3PO_4$ (85%) and dil. to 500 ml and mix.

(b) *Alkaline copper soln.*—Dissolve 40 g anhyd. $Na_2CO_3$ in ca 400 ml $H_2O$ and transfer to liter flask. Dissolve 7.5 g tartaric acid in this soln and then 4.5 g $CuSO_4 \cdot 5H_2O$; mix and dil. to vol. If sediment forms on standing decant and use clear supernatant liquid.

(c) *Standard dextrose soln.*—(1) *Stock soln.*—Dissolve 1.0 highest purity anhydrous dextrose in ca 50 ml filtered 0.25% benzoic acid soln and dil. to vol. in 100 ml volumetric flask with 0.25% benzoic acid soln. This standard keeps indefinitely.

(2) *Dilute standard soln.*—Transfer 20 ml of the stock soln (c) (1) to 100 ml volumetric flask, and make to vol. with water (or 0.25% benzoic acid soln if the soln is to be kept for any length of time). Mix well; 1 ml is equivalent to 100 mg % dextrose.

#### DETERMINATION

Transfer 2 ml of the soln to be analyzed to a Folin-Wu blood sugar test tube. Add 2 ml alkaline copper soln. The surface of the mixture must now have reached the constricted part of the tube. Transfer the tube to a boiling water bath and heat for 6 min. Transfer to a cold water bath and let cool, without shaking, for 2–3 min. Add 2 ml phosmolybdic acid soln. When the cuprous oxide is dissolved, dil. the soln to the 25 ml mark with more of the phosmolybdic acid soln dild 1:4, insert a rubber stopper, and mix. Allow to stand 10–15 min. Transfer a portion to a colorimeter tube and read within the next 15 min. in a photoelectric colorimeter at 420 mμ. Run parallel determinations on 2 ml distilled water to be used as a blank in the colorimeter, and on 2 ml of standard dextrose soln to be read against the blank set at 0.

$$\text{Calculation: } \frac{\text{conc. standard}}{\text{transmittance standard}} \times \text{transmittance unknown} = \text{conc. unknown.}$$

(2) The following table of refractive index values for raffinose hydrate was adopted:

*Refractive indices of raffinose hydrate solutions at 20°C.**

| RAFFINOSE HYDRATE, BY WEIGHT IN AIR PER CENT | REFRACTIVE INDEX | RAFFINOSE HYDRATE, BY WEIGHT IN AIR, PER CENT | REFRACTIVE INDEX | RAFFINOSE HYDRATE, BY WEIGHT IN AIR, PER CENT | REFRACTIVE INDEX | RAFFINOSE HYDRATE, BY WEIGHT IN AIR, PER CENT | REFRACTIVE INDEX |
|---|---|---|---|---|---|---|---|
| 0 | 1.33299 | 10 | 1.34576 | 20 | 1.35953 | 30 | 1.37424 |
| 1 | 1.33422 | 11 | 1.34709 | 21 | 1.36096 | 31 | 1.37577 |
| 2 | 1.33546 | 12 | 1.34844 | 22 | 1.36239 | 32 | 1.37730 |
| 3 | 1.33671 | 13 | 1.34979 | 23 | 1.36384 | 33 | 1.37884 |
| 4 | 1.33797 | 14 | 1.35116 | 24 | 1.36529 | 34 | 1.38040 |
| 5 | 1.33924 | 15 | 1.35253 | 25 | 1.36676 | 35 | 1.38197 |
| 6 | 1.34052 | 16 | 1.35391 | 26 | 1.36824 | 36 | 1.38356 |
| 7 | 1.34181 | 17 | 1.35530 | 27 | 1.36972 | 37 | 1.38516 |
| 8 | 1.34311 | 18 | 1.35670 | 28 | 1.37121 | 38 | 1.38677 |
| 9 | 1.34443 | 19 | 1.35811 | 29 | 1.37272 | 39 | 1.38840 |
| | | | | | | 40 | 1.39004 |

* Zerban and Martin, *J. Assoc. Official Agr. Chem.*, 34, 808 (1951).

## 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 following method for propylthiouracil was adopted, first action:

Start and complete determination in one day.

Transfer to a 200 ml vol. flask an accurately weighed quantity of sample representing 150 mg of propylthiouracil. To another 200 ml vol. flask transfer 150.0 mg of propylthiouracil standard. To each flask add 150 ml of $NH_4OH$ soln (1+13) (ca 2% $NH_3$), washing down necks. Shake sample flask moderately, continuously, 1 min. to dissolve propylthiouracil. Shake standard flask moderately until powder is dissolved. Dilute both to mark with $NH_4OH$ soln (1+13) and mix.

Filter sample liquid, rejecting first 25 ml of filtrate. Accurately dilute a 20 ml aliquot of clear filtrate with $H_2O$ to 200 ml in a vol. flask (or 25 to 250) and mix. Accurately dilute a 20 ml aliquot of resulting soln to 200 ml in a vol flask (or 25 to 250) and mix. Make same accurate double dilution of standard soln, using same quantities. (Final concn. 0.0075 mg/ml.)

Measure optical density of final soln of standard and of sample, each against an $H_2O$ blank, in silica cells in a spectrophotometer at 234 m$\mu$. Apply cell corrections unless same cell is used successively for standard and sample. Compute propylthiouracil content of sample.

(2) The following microchemical tests were adopted, first action.

DIPHENHYDRAMINE HYDROCHLORIDE (BENADRYL HYDROCHLORIDE ®)

*Reagents.*—5% aqueous solution of platinic chloride ($H_2PtCl_6 \cdot 6H_2O$); glycerin-alcohol soln (1+1).

*Preparation of sample.*—Add a small amount of the pure substance or tablet material (≈0.2 mg.) to 1 drop of glycerin-alcohol on a microscopical slide and stir into the drop; or prepare an aqueous soln of the substance.

*Identification.*—To a drop of the test soln, draw in 1 drop of platinic chloride reagent. Aggregates of platy crystals form readily in glycerin-alcohol medium and gradually in the aqueous test drop if Benadryl ® is present. The plates show jagged edges and have a tendency to twin, forming X-shaped aggregates, hour-glass forms, and dendritic structures. The crystals show first order gray polarization colors and exhibit symmetrical or parallel extinction with respect to the various forms of crystals produced. The plates show positive elongation.

*Sensitivity.*—1:20,000.

PYRANISAMINE MALEATE

*Reagents.*—5% aqueous soln of platinic chloride ($H_2PtCl_6 \cdot 6H_2O$); glycerin-alcohol solution (1+1).

*Preparation of sample.*—Add a very minute amount (ca. 0.1 mg) of the substance to 1 drop of glycerin-alcohol or distilled water on a microscope slide, and stir into the drop.

*Identification.*—To a drop of the test soln, draw in 1 drop of platinic chloride reagent, and stir. (Or the dry substance may be added directly to a drop of the reagent.) Needles are formed in rosette aggregates, sheaves, and singly. The needle crystals show second order blue and green and first order red and yellow polarization colors and exhibit parallel extinction and negative elongation.

*Sensitivity.*—1:20,000.

TRIPELENNAMINE HYDROCHLORIDE (PYRIBENZAMINE HYDROCHLORIDE ®)

*Reagents.*—5% aqueous soln of platinic chloride ($H_2PtCl_6 \cdot 6H_2O$); glycerin-alcohol soln (1+1).

*Preparation of sample.*—Add a very minute amount of the pure substance or tablet material to 1 drop of glycerin-alcohol or distilled water on a microscope slide, and stir into the drop.

*Identification.*—To a drop of the test soln, draw in 1 drop of platinic chloride reagent, and stir. Small needles and bladed crystals are formed in dense rosette aggregates and singly. The needles show white and first order yellow polarization colors and exhibit parallel extinction and positive elongation.

*Sensitivity.*—1:20,000.

## 33. COSMETICS

(1) The following method for methanamine was adopted, first action:

REAGENTS

*Sodium bisulfite.*—10% w/v and 0.5% w/v.

*Iodine.*—0.05 *N*.

*Starch indicator soln.*— 5.3(e).

*Borax-carbonate soln.*—Dissolve 5.0 gm $Na_2CO_3$ and 4.0 gm $Na_2B_4O_7 \cdot 10H_2O$ in 100 ml of water.

PROCEDURE

Pipet an aliquot containing 150–200 mg. of methenamine into a 500-ml round-bottom flask and dilute to 30 ml with water. Neutralize to litmus with either

NaOH or $H_2SO_4$, then acidify with 1 ml of conc. $H_2SO_4$. Connect the flask to a water-cooled condenser and heat under reflux for 30 min. to hydrolyze the methenamine. Carefully add 175 ml of water thru the top of the condenser, and disconnect the condenser from the round-bottom flask. Connect the flask thru a Kjeldahl trap to an efficient straight-wall condenser, and distill the contents into a 200 ml volumetric flask containing 10 ml of a freshly prepared 10% $NaHSO_3$ soln. Continue the distillation until the residual volume is about 5 ml, taking care to avoid charring. Wash down the condenser with a little water and cool the distillate to room temp. Dilute the distillate to the mark with water, mix well, and allow to stand for 30 min. Pipet a 20 ml aliquot into a wide-mouth 250 ml Erlenmeyer flask, add 3–4 ml of starch indicator soln and destroy the excess bisulfite with ca 1 *N* iodine. Carefully adjust to the starch-iodine end point with 0.5% $NaHSO_3$ and 0.05 *N* iodine. Dilute to 50 ml with water, add 10 ml of borax-carbonate soln and titrate with 0.05 *N* iodine until a permanent blue color is produced.

1 ml 0.05 *N* Iodine = 0.584 mg methenamine.

(2) The following method for phenolsulfonates was adopted, first action:

REAGENTS

*Potassium bromate, 0.1 N.*—Dissolve 2.8 gm potassium bromate in 1000 ml of water. Standardize against 0.1 *N* sodium thiosulfate.

*Sodium thiosulfate, 0.1 N.*

*Starch indicator soln.*—5.3(e).

PROCEDURE

Pipet an aliquot containing 60–125 mg of phenolsulfonic acid into a 250 ml iodine flask and dilute to ca 75 ml with water. Add 2–3 ml of conc. HCl, 2–3 g of KBr, and titrate slowly with 0.1 *N* $KBrO_3$ until an excess of 1–3 ml is present. (In the early stages of the titration the bromine formed disappears rapidly but near the end point some time is required for the bromine to react.) Stopper the flask and let stand for 10 min. If the color disappears, add more 0.1 *N* $KBrO_3$ and let stand for another 10 min. Add 2–3 g KI, shake thoroly, and titrate the liberated iodine with 0.1 *N* $Na_2S_2O_3$ using starch as indicator. The iodine liberated is equivalent to the excess of $KBrO_3$ soln added. From the net volume of $KBrO_3$ soln required in the bromination reaction, calculate the amount of phenolsulfonic acid in the sample.

1 ml 0.1 *N* $KBrO_3$ = 0.00435 g phenolsulfonic acid.

(3) The following qualitative and quantitative methods for the examination of thioglycolate solutions were adopted, first action:

*Qualitative Test*

Dilute 2 ml of sample to 10 ml with water, acidify with 10% acetic acid, adding 5 ml in excess and shake well. Add 2 ml of 10% cadmium acetate and shake. A white gelatinous precipitate forms if thioglycolic acid is present. Add an excess of 10% ammonium hydroxide and shake. The precipitate of cadmium thioglycolate will dissolve.

*Quantitative Determination*

REAGENTS

*Methyl red indicator soln.*—2.46(c).

*Starch indicator soln.*—5.3(e).

*Iodine, 0.1 N.*

PROCEDURE

Pipet an aliquot of the sample containing 250–300 mg thioglycolic acid into a wide-mouth 250-ml Erlenmeyer flask. Dilute to 50 ml with water, add 2–3 drops of methyl red indicator soln, and make slightly acid with concentrated HCl. Add 3–4 ml of starch indicator soln and titrate with 0.1 *N* iodine to a purple end point.

1 ml of 0.1 *N* iodine = 0.009209 g thioglycolic acid.

Reducing substances, other than thioglycolic acid, will interfere with the method.

(4) The following qualitative tests for potassium bromate and sodium perborate were adopted, first action:

**(1)** Note the physical characteristics of the product.
  **(a)** $KBrO_3$ and $NaBO_3$ are white crystalline salts soluble in water.
**(2)** Check the *p*H of an aqueous soln with *p*H test paper.
  **(a)** $KBrO_3$ in aqueous soln is slightly acid.
  **(b)** $NaBO_3$ in aqueous soln is strongly alkaline.
**(3)** Make a flame test in a slightly darkened room using a platinum wire.
  **(a)** When viewed thru a cobalt glass $KBrO_3$ gives a reddish-violet flame.
  **(b)** $NaBO_3$ gives the typical yellow sodium flame.
**(4)** Test for an oxidizing agent. Dissolve 0.1 g of neutralizer in 10 ml of water, acidify with conc. HCl and add 0.5 g KI.
  **(a)** $KBrO_3$ and $NaBO_3$ liberate iodine.
**(5)** If tests **1, 2a, 3a,** and **4** are positive for $KBrO_3$ make the following test for bromine: To 1 ml of a 5% aqueous soln of the neutralizer in a test tube slowly add 2 ml of conc. $H_2SO_4$ with vigorous shaking. Note with *caution* the odor and color of the liberated gas. Cool the test tube. *Carefully* add 2 ml of $CS_2$ and shake. The carbon disulfide layer becomes yellow or red if bromine is present.
**(6)** If tests **1, 2b, 3b,** and **4** are positive for $NaBO_3$ make the following test for boron. Moisten 0.2 g of neutralizer with 1–2 drops of conc. $H_2SO_4$, add 2 ml of methyl alcohol, stir well, and ignite. A green flame indicates boron.

## 34. COLORING MATTERS

(1) The following method for sulfonated amine intermediates in coal-tar colors Lake Red C Amine in D&C Red Nos. 8 and 9, was adopted, first action:

REAGENTS

*Standard solution of Lake Red C Amine (10 mg/l).*—Dry a purified sample of sodium salt of Lake Red C Amine for four hours at 105°C. Transfer 100 mg of the amine to a 200 ml volumetric flask and add ca 150 ml of water. When all the amine is dissolved, make to volume with water and mix well. Transfer a 10 ml aliquot to a 500 ml volumetric flask and dilute to ca 450 ml with water and make the standard soln slightly alkaline (*p*H about 8) with (1+1) ammonium hydroxide. Dilute to the mark with water and mix thoroly.

*$BaCl_2$.*—2% in water.

PROCEDURE

Transfer a 1.0 g sample of the dye to a 500 ml tall-form beaker. Wet the sample with 5 ml of acetone and then add 100 ml of 2% $BaCl_2$ soln. Boil the mixture for 10 min. and filter the hot mixture thru a Whatman No. 12 folded filter paper into a 500 ml Pyrex separator. Return the filter paper and the dye slurry it contains to the original beaker, repeat the boiling water extraction and filter as before. Make a

third hot water extraction in the same manner. Discard the filter paper and dye slurry.

Cool the combined filtrates, acidify with 5 ml of dilute HCl (1+1) and extract with three 20 ml portions of benzene. Wash the combined benzene extracts with a 20 ml portion of water and add this wash water to the pooled filtrates.

Insert a cotton plug into the stem of the separator and filter the soln into a 500 ml beaker. Add boiling chips to prevent bumping and boil the soln 15–20 min. to remove the benzene. Cool the soln and adjust the *p*H to about 8.0 with dilute $NH_4OH$. Transfer the alkaline soln to a 500 ml volumetric flask, dilute to the mark with water and mix thoroly.

Determine the absorbancy of the sample and the standard at 247 mμ. Calculate the per cent Lake Red C Amine in the sample by means of the following equation:

$$\% \text{ Lake Red C Amine} = \frac{A_{Un} \times 0.91C \times 100}{A_{Std} \times W}.$$

Where $A_{Un}$ = Absorbancy of the sample at 247 mμ.
$A_{Std}$ = Absorbancy of the standard at 247 mμ.
$C$ = Concentration of the standard in milligrams per liter.
$W$ = Weight of sample in milligrams.

(2) The following method for subsidiary dyes in FD&C colors—lower sulfonated dyes in FD&C Yellow No. 5, was adopted, first action:

REAGENTS

*Isoamyl alcohol.*—Reagent grade.
*Petroleum ether.*—Reagent grade.
*Hydrochloric acid.*—Conc.
*Hydrochloric acid.*—Ca. 0.25 *N*.

*Spectrophotometric Standard*

Either **(a)** the purified disodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-phenylazopyrazole, or **(b)** purified FD&C Yellow No. 5 (recrystallize twice from (1+1) alcohol-water; dry at 135°C.) may be used as the standard.

Dissolve 100 mg of the standard dye in 1000 ml of $H_2O$. Transfer a 10 ml aliquot of this soln to a 100 ml volumetric flask, add ca 1 g of solid ammonium acetate and dilute to volume with water.

For Standard **(a)** $$\% \text{ Subsidiary} = \frac{\text{Absorbancy of sample at 434 m}\mu}{\text{Absorbancy of standard at 434 m}\mu}$$

For Standard **(b)** $$\% \text{ Subsidiary} = \frac{\text{Absorbancy of sample at 434 m}\mu}{1.1 \times \text{Absorbancy of standard at 434 m}\mu}.$$

APPARATUS

A spectrophotometer capable of measuring absorbancy at 434 mμ.

PROCEDURE

Prepare a soln containing 200 mg of the color in 100 ml of $H_2O$ (heat on steam bath if necessary for complete soln). To 50 ml of this soln add 1 ml of HCl and extract the lower sulfonated dye by shaking the soln successively in 3 separatory funnels, each containing 50 ml of amyl alcohol. Wash the amyl alcohol extracts by shaking successively with 50 ml portions of 0.25 *N* HCl, until the washings are practically colorless, passing each acid portion thru the funnels in the order used for the original amyl alcohol extraction. Dilute the amyl alcohol extracts in each

funnel with 1–2 volumes of petroleum ether and remove the lower sulfonated dye by washing with several 10–20 ml portions of water, passing each portion thru the 3 funnels in the reverse order to that previously followed. Transfer the extracted color to a 100 ml volumetric flask, add ca 1 g of solid ammonium acetate, dilute to volume with water, and measure the absorbancy of the unknown soln and the standard soln at 434 mμ using 1 cm cells.

### 35. EXTRANEOUS MATERIALS—ISOLATION

(1) The following method for light filth in Spanish peanuts was adopted, first action:

Transfer 100 g sample of peanuts to a 2 liter trap flask. Add 250 ml Tween 80–60% alcohol soln (40 ml Tween 80 + 210 ml 60% alchohol). Mix well and add 60% alcohol to make 800 ml volume. Trap off twice in 60% alcohol with 75 and 40 ml of gasoline, respectively, in the usual manner. Except for occasional circular upward strokes within the first 20 min. after the flask is filled with 60% alcohol, avoid stirring during the remainder of the standing period. Allow each of the two extractions to stand 1½ hrs. Use 60% alcohol in rinsing the neck of the flask. Filter each gasoline extract separately, and examine filters at ca 30×.

(2) Procedure **35.23** (p. 712) for filth in shredded coconut was deleted and the following method was adopted, first action:

**(a)** *Heavy filth.*—Proceed as directed under **35.32** using 100 g sample in 400 ml beaker.

**(b)** *Light filth.*—Weigh 100 g of sample into a 2 liter beaker. Add 1 liter hot 5% borax soln. Boil 10–15 min. Pour thru 8″ No. 140 sieve. Wash well with hot water. Using a wide aperture, stemless funnel, transfer the coconut to a 2 liter Wildman trap flask with ca 700 ml 60% alcohol. Wash the sieve with a forcible stream of hot water, collecting the final residue at one edge of the screen and transferring to the trap flask with a stream of 60% alcohol. Add 50 ml of gasoline to the trap flask and mix thoroly. Fill the flask with 60% alcohol. Allow to stand 30 min. with occasional gentle beating and lifting of the coconut material to free any rodent hairs adhering to it. Trap off the gasoline layer, using 60% alcohol as rinse, and filter thru ruled filter paper. Add 25 ml of gasoline to the trap flask and make a second extraction. After 30 min. trap off and filter on a second paper. Count filter papers at ca 30×.

(3) Sections **35.2(d),** Pancreatin soln (p. 702), and the first 3 paragraphs of **35.4(a),** Operation of Wildman trap flask (p. 704), were deleted and the following paragraphs were substituted:

**35.2(d)** *Pancreatin Soln (should be freshly prepared).*

Mix 5 g of U.S.P. Pancreatin with 100 ml of warm $H_2O$ (not over 40°). Stir with a malted milk mixer for 10 min., or allow to stand 30 min. with intermittent stirring. Pour soln thru a 4″ loosely packed pad of cotton in a 4 or 5″ 60° funnel. Repeat filtration thru same pad. If filtering is slow in either filtration, change cotton. Filter with suction thru rapid-action paper in a Büchner or Hirsch funnel. If filtration is not rapid pour the soln thru a slightly compressed cotton plug in the 60% funnel. Repeat if necessary until rapid filtration thru paper is obtained.

**35.4(a)** *Operation of Wildman trap flask.*

Place the liquid-food mixture in the flask and make the volume of liquid ca 900 ml (2 liter trap flask) or ca 600 ml (1 liter trap flask). Add gasoline or oil as stated in specific method. Tilt the flask about 45° from vertical and mix with a brisk rotary

motion so that the liquid is brought to a roll. Avoid splashing thru the surface of the liquid with the rubber stopper. Use 400–500 strokes during a 2 min. stirring period. Add sufficient liquid to bring oil layer well into the neck of the flask. Unless otherwise stated, allow mixture to stand 30 min., with intermittent stirring of the bottom layer every 3–6 min. during first 20 min. of the standing period. Trap off by raising stopper as far as possible, being sure that all the oil layer and at least 1 cm of liquid below are above the stopper. Spin stopper while raising it to remove the food particles which are on it. Hold stopper in place and decant. Rinse out material on the rod and in the neck of the flask with $H_2O$ or liquid in which flotation was carried out. Using suction, filter trapped material and rinsings thru a rapid-action fiction filter paper in a Hirsch or Büchner funnel.

Add to trap flask gasoline or oil as specified, and stir vigorously. Add sufficient flotation liquid to bring the floating layer into the neck of the flask. Trap off, using same technique as before.

(4) The following changes were made in the first action method for insect fragments and rodent hairs in baked products, prepared cereals, and alimentary pastes, **35.28** (p. 714), and the revised method was adopted, first action:

**35.28(a),** third paragraph, line 6, insert "60%" before alcohol."

**35.28(b),** line 4, delete "ca neutralize with NaOH soln," and substitute "neutralize to ca *p*H 6 with dil. NaOH soln."

(5) The first action pancreatic digestion method for insect fragments and rodent hairs in white wheat flour, **35.29(a)** (p. 714), was revised as follows and adopted, first action:

Weigh 50 g of well mixed flour into a beaker, add ca 300 ml of water and stir rapidly into a smooth, thin slurry. Add 50 ml of pancreatin soln **35.2(d)**. Adjust mixture to *p*H 8 with dilute $Na_3PO_4$ soln. Add water to bring the volume up to 400 ml. Readjust *p*H after 15 min. and again after 45 min. After 30 min. stir in 3 drops of formaldehyde and digest for 16–18 hours at 30–37°.

Transfer digested material to a 2 liter trap flask. Proceed as in section **35.4(a)** (as revised above). Catch combined trappings and rinsings in a beaker and transfer to a 2 liter trap flask. Trap off as above. Filter directly or, if starchy material is present, acidify to 1–2% with HCl, bring to a boil, and then filter as in **35.4(a)**. Examine at ca 30×.

(6) The following change was made in the first action method for insects, insect parts, and rodent hairs in whole wheat flour, **35.36(d)** (p. 716), and adopted as first action:

Change "**35.29**" to "**35.33.**"

(7) The following change was made in the first action method for filth in starch, **35.37** (p. 716), and adopted as first action:

Third line, change "80%" to "60%."

(8) The first action method for rot in apple butter, **35.48** (p. 719), was adopted as official.

(9) The first action method for mold in frozen strawberries, **35.55** (p. 721), was adopted as official.

(10) The following methods for rot in frozen and canned drupelet berries were adopted, first action:

*Blackberries, Raspberries, and Other Drupelet Berries (Frozen)*

Pulp thawed berries thru cyclone with screen openings 0.027″ in diam. and mix thoroly. Mix 25 g of pulp with 50 g of 3% pectin soln **35.2(b)**. Make mold count as directed in **35.64**.

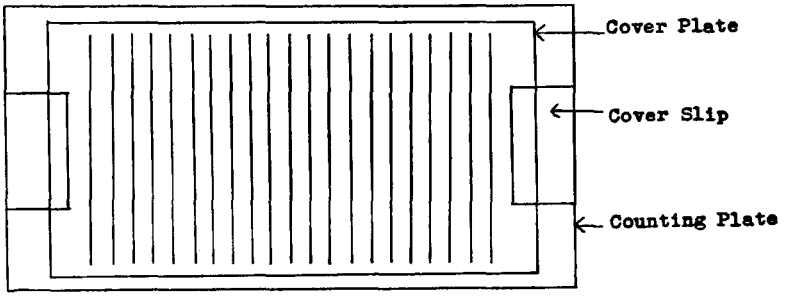
*Blackberries, Raspberries, and Other Drupelet Berries (Canned)*

Drain the berries 2 min. on a No. 20 sieve. Pulp, dilute, and make mold count as directed for frozen berries.

(11) The following method for rot fragments in tomato products was adopted, first action:

IDENTIFICATION OF TOMATO ROT FRAGMENTS

*Counting plate and cover for rot fragments.*—Plates are of glass 1.5–4.0 mm thick and covers are ca 1.5 mm thick. Dimensions of plates 55×100 mm; rulings: crosswise, parallel lines 4.5 mm apart, with one 15 mm space at each end. One-half of square cover-slip ca 22 mm on a side and ca 0.25 mm thick is fastened at each end of counting plate by balsam to separate it from the cover-plate. Covers are 50 mm×85 mm.




Rot Fragment Counting Slide

Weigh 10 g of juice (5 g of puree or catsup or 2 g of paste) and transfer with 100 ml of $H_2O$ to 400 ml beaker. Add ca 2 ml of saturated aqueous gentian violet soln (10% gentian violet in alcohol may replace the aqueous soln when latter fails), stir, and allow to stain 3 min. Add ca 200 ml of $H_2O$, stir, and pour thru No. 60 sieve ca 7.5 cm in diam., held in horizontal position. Pour material over entire surface of sieve, using glass rod held against lip of pouring beaker, with lower end of rod ca 2 cm from screen. If sample weight specified does not drain rapidly, reduce size of sample. Rinse beaker with 200 ml of $H_2O$ and pour rinse $H_2O$ over tomato debris on sieve, using glass rod as before. Tilt sieve to ca 30° angle and wash debris to lower part with ca 100 ml of $H_2O$. Allow debris to drain and transfer to bottom of graduated tube, ca 12×3 cm, by means of spatula. Transfer remaining debris by washing down with water from a dropper and immediately taking up debris in wash water before it has run thru the screen. When completely transferred, make volume of $H_2O$ and debris to 10 ml with $H_2O$. Add sufficient pectin soln **35.2(b)** to bring vol-

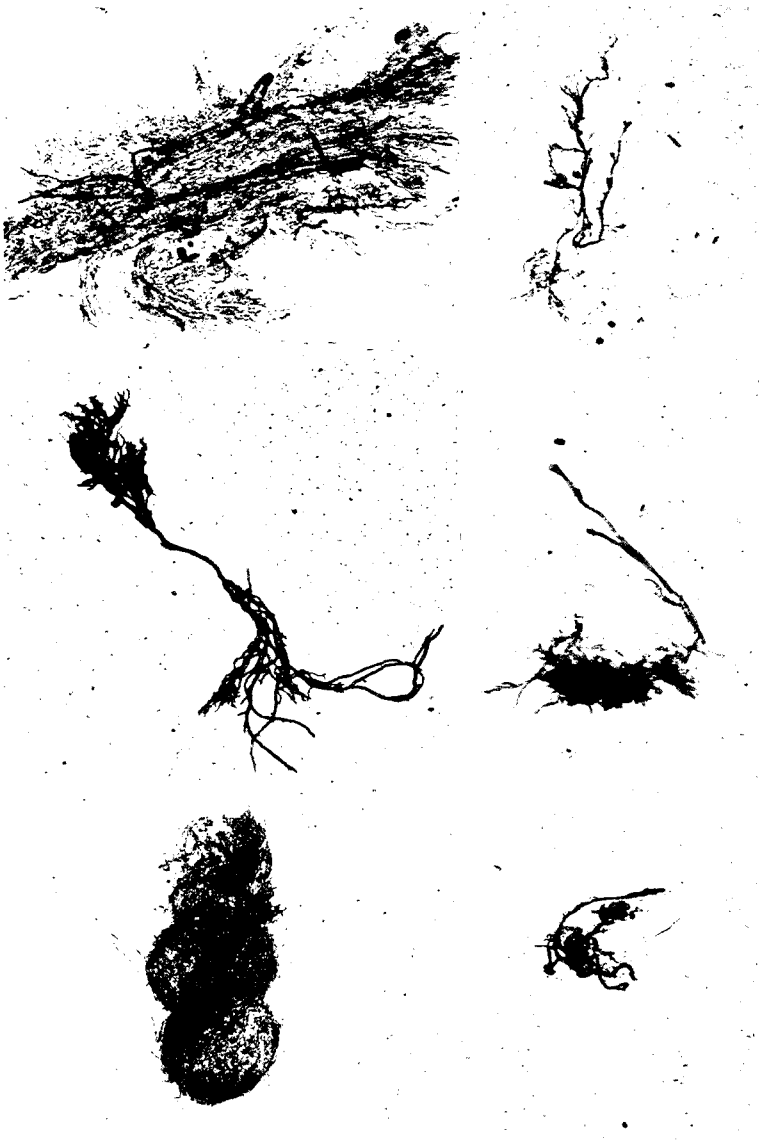


Plate 1.—Rot Fragments from Tomato Puree. 40× (before reduction).

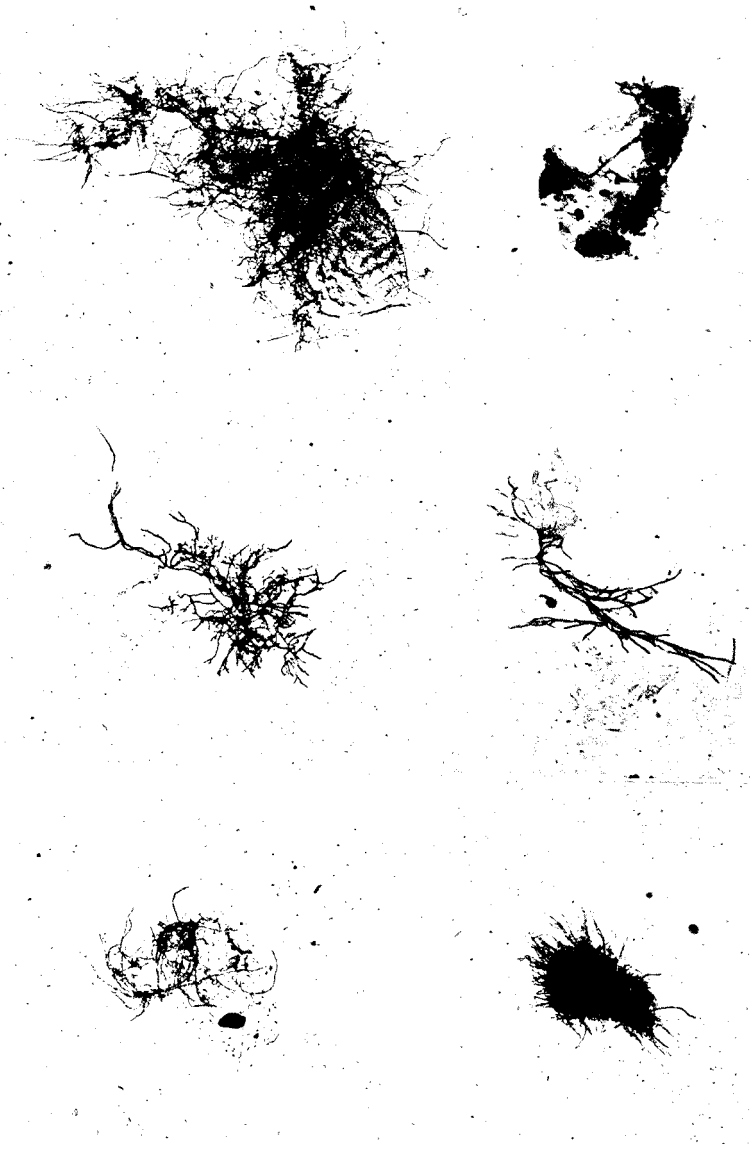


Plate 2.—Rot Fragments from Tomato Puree. 40× (before reduction).

ume to 20 ml. Mix stained suspension well, measure out two separate 0.5 ml portions, spread over 2 counting slides, and cover with special cover slip. Examine each slide with Greenough-type microscope **35.1(k)**, using magnification of 40–45 diam. with transmitted light. (Rot fragments are tomato tissue to which mold filaments are attached. Some may appear as almost solid masses of mold. (See Plates.)) Count number of rot fragments on each of the 2 slides, add, and multiply by 2 (10 g sample), 4 (5 g sample), or 10 (2 g sample) to obtain number of rot fragments/g of product.

(12) The first action method for filth in sweet corn, **35.80(a)** (p. 729), was modified as follows and adopted, first action:

(a) Line 7, insert after end of sentence " . . . nearly to boiling," the following:

"Transfer contents of beaker onto a No. 200 sieve, wash beaker thoroly with hot alcohol to insure complete transfer. Wash material on sieve with hot alcohol from a wash bottle to eliminate oily mass."

(b) Line 8, change "Pour oil and $H_2O$ mixt." to "transfer residue."

(c) Line 10, delete "to dissolve oil and speed filtration."

(13) The following method for preparing standard sediment disks was substituted for the first action method **35.9(c)** (p. 706) and adopted, first action.

**(c)** *Preparation of standard sediment disks.*—Make a uniform mixture of oven dried (100°) materials which meet following screening specifications, using U. S. Standard Sieves. Grind all materials by hand with mortar and pestle.

| | *per cent* |
|---|---|
| Cow manure, thru No. 40 | 53 |
| Cow manure, thru No. 20, retained on No. 40 | 2 |
| Garden soil, thru No. 40 | 27 |
| Charcoal, thru No. 40 | 14 |
| Charcoal, thru No. 20, retained on No. 40 | 4 |

Place 2.00 g of above mixture in 100 ml volumetric flask and moisten with 5 ml 1% aerosol soln or other suitable wetting agent, add 46 ml 0.75% gum soln such as carob bean **(35.2(c))**, bring level of liquid just into neck of flask by adding 50% by weight sucrose soln, let stand at least 30 min., add few drops of alcohol and dilute to volume with the sucrose soln. Mix thoroly, pour into 250 ml beaker or 6–8 fl. oz. screw-capped jar, and stir with small motor stirrer at speed (ca 200–300 r.p.m) such that mixture is thoroly agitated, but so that very little air is whipped into the suspension. Observe with light. Place blade of stirrer so that fine particles do not accumulate in small eddies at bottom of beaker.

Transfer (while stirring) 10 ml portion (200 mg standard sediment) with large tipped, graduated pipet to a volumetric flask, and make up to one liter with 50% by weight sucrose soln. When thoroly mixed, each ml contains 0.2 mg sediment. Mix contents of flask, pour into 1500 ml beaker, and stir with suitable motor stirrer as directed above. If particles accumulate on side of beaker, wash down with portions of sediment suspension or push under with tip of pipet. While stirring, pipet definite volumes of sediment mixture and add to ¾ pint filtered sweet skimmed milk. Mix thoroly and pass mixture thru standard sediment disk in filtering device having filtering area measuring 1¾" in diameter. Pour milk gently down side of filtering apparatus and filter with very little or no suction. Wash container promptly with

¼ pint filtered skimmed milk. Let last portion of milk flow thru pad with no suction applied. If sediment does not appear to be evenly distributed over pad, add 15 or 20 ml milk and let it filter thru without any suction. Repeat this until sediment appears to be evenly distributed. Suck air thru disk for ca 1 min. to remove excess milk. For permanent record mount and spray disks with 40% HCHO soln or with alcoholic soln containing 2.5 g each of menthol and thymol in 100 ml. Alternatively, if most of milk is removed by thoro aspiration, no preservative is needed. Dried pads may be coated with colorless plastic cement diluted with 1–3 volumes of acetone so that mixture is thin enough to pour easily. If acetone dissolves pigment from paper and stains pads, place them on flat glass plate for treating with diluted cement. Move pads while drying to prevent sticking to glass. When pads are almost dry place a light weight (petri dish, etc) directly on them to prevent curling. Pads may be mounted with the plastic cement. (Standard disks made from manure containing large amount of chlorophyll cannot be coated with plastic cement, as solvent will extract chlorophyll and stain pad green. Use this method of preserving pads only if there is no leaching of pigment from the sediment on addition of diluted plastic cement.) Following above method, prepare series of disks containing sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, and 14.0 mg standard mixture. Mark disks to show quantity of sediment (mg) used to prepare each pad. Do not use as a standard any pad on which sediment is not evenly distributed.

For purpose of comparison, entire series of disks may be used, but it will usually be found more convenient to select a few disks denoting variations in grade that are applicable to particular investigation being made. If grading charts are prepared and reports made, chart and report must indicate whether mixed or off-bottom sample was used. If standards are to be handled or used for any appreciable length of time, place them under glass, transparent plastic sheets, or other suitable materials. In using standards, grade sediment disk of sample to nearest standard disk regardless of whether actual quantity of sediment is above or below standard. Disregard gross pieces of material (whole flies, large chunks of manure, etc.) in grading pads, but list their presence separately.

(14) The following method for urea by urease-bromothymol blue test paper was adopted, first action:

*Qualitative Test—First Action*

(Applicable to cloth or sack fibers, whole or ground cereal grains, whole or chopped nuts, spices, neutral solutions, etc.)

REAGENTS

**(a)** *Urease soln.*—0.2 g urease powder per 10 ml $H_2O$. Wet powder with small amount of $H_2O$ and stir into a paste before diluting to volume.

**(b)** *Bromothymol blue soln.*—0.15 g bromothymol blue per 50 ml. Rub 0.15 g indicator powder in a mortar with 2.4 ml of 0.1 *N* NaOH soln. After indicator has dissolved wash the mortar and pestle and dilute soln to 50 ml. The soln should be green, which indicates ca *p*H 7.0.

**(c)** *Test paper A.*—Mix 10 ml of indicator soln **(b)** with 10 ml of urease soln **(a)**. Pour mixture into a watch glass. Using clean tweezers, dip pieces of heavy filter paper (Whatman No. 5, CS&S No. 598, or 589 green ribbon have been found satisfactory) in the mixed urease-indicator soln. (To avoid uneven distribution of indicator and enzyme wet the whole piece of paper at one time by laying it onto the surface of the mixed solns.) Hang the paper to dry in a place free from ammonia fumes, strong air currents, or heat. The paper should be orange colored when dry. Store the dry paper in a well stoppered, dark glass bottle in a cool place.

(d) *Test paper B.*—Dilute indicator soln (b) with an equal portion of water. Dip pieces of filter paper (same kind as used for test paper A) in the indicator soln and hang to dry similarly to test paper A.

DETERMINATION

Neutral solutions can be tested for urea by placing a drop on dry test paper A (c). The appearance of a blue or green color after a few min. incubation at room temp. indicates urea.

For the detection of urea in very small dry particles, dip pieces of both test papers A (c) and B (d) of appropriate size into water using clean tweezers. Wet each whole paper at one time by laying it onto the surface of the water (the indicator flows unevenly if the paper is wet with drops). Shake each paper to remove excess water and lay it onto a clean flat piece of glass. (If paper on glass has shiny appearance too much water has been added. Allow to dry slightly before using.) Place sample on the papers, cover with another clean, flat piece of glass and press down gently.

Immediate development of blue color on both papers A and B is indicative of alkaline particles. If the alkaline particles are extremely small color development is delayed 10–30 seconds, but will develop on both papers. Blue spots which develop on test paper A alone are indicative of urea. Urea in the sample is hydrolyzed by the urease in the paper to yield ammonia which changes the bromothymol blue to a blue spot. The reaction under conditions of the test requires 30–60 seconds to give detectable color, the time varying inversely with the urea concentration. The spots continue to develop and enlarge for 10–20 minutes and then fade gradually.

Larger particles may be tested similarly to the very small ones. However, depending on shape and size of particles it may not be practical to cover them with a glass plate. The papers must be protected from $NH_3$ fumes and from drafts that would remove the $NH_3$ liberated from the urea. Interpretation is the same as for small particles.

## 36. MICROBIOLOGICAL METHODS

No additions, deletions, or other changes.

## 37. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

## 38. RADIOACTIVITY

No additions, deletions, or other changes.

## 39. STANDARD SOLUTIONS

No additions, deletions, or other changes.

## 40. VITAMINS

(1) The first action method for vitamin A in mixed feeds, *This Journal*, **34**, 97–99 (1951), was modified as follows and adopted, first action:

(a) Under "Apparatus" on page 97, line 9, delete "Soxhlet" and add "any suitable ground-glass joint refluxing apparatus."

(b) Under "Reagents" on page 98, line 2, after description of "Adsorbent" add "(See Note 1)" and append the following to the procedure:

NOTE 1: Check the adsorbent for recovery of vitamin A by drawing thru the described adsorption column with suction 50 ml of a hexane soln containing a

known number of units of true vitamin A ester (25–40 units) plus ca 100 micrograms of beta carotene (obtainable from General Bio-Chemicals, Inc., 677 Laboratory Park, Chagrin Falls, Ohio). As to chromatography and colorimetry follow the prescribed procedure. Recovery should be 90–100 per cent. If the adsorbent is too retentive it may be conditioned by allowing a thin layer (2 or 3 mm. thick) of the magnesia (prior to mixing with Super-Cel) to be exposed to air for 60–70 hours, or a recovery factor for the adsorbent used may be employed in final calculation of results.

(c) Under "Reagents" page 98, line 8, after description of "Antimony trichloride reagent" add "(See Note 2)" and append the following to the procedure:

NOTE 2: In preparing this reagent, the use of a fresh bottle of antimony trichloride crystals which has not been opened previously is desirable. The crystals should possess a translucent appearance. Crystals which have been stored too long or are kept in a bottle which has been opened, frequently contain an objectionable brown-colored decomposition material. The antimony trichloride plus the prescribed volume of chloroform are merely heated on a hot plate until solution takes place. The solution is cooled and 3 per cent of acetic anhydride is mixed in. The reagent thus prepared usually is clear; if not, it may be easily centrifuged, filtered through glass wool or allowed to settle and decanted. It may be stored in a brown bottle for several months.

(d) Under "Procedure" on page 98, line 9, after description of "Extraction" add "(See Note 3)" and append the following to the procedure:

NOTE 3: Although beta carotene predominates in practically all feed ingredients, particularly alfalfa meal, there is some alpha carotene present and alpha carotene is eluted from a magnesia adsorption column before beta carotene. Investigations indicate that the colorless vitamin A band, although eluted before the beta carotene band, may not be all eluted before the alpha carotene band. Therefore, the usually very light alpha carotene band must be all eluted and a small amount of the heavy beta carotene band must be washed from the column in order to pass the vitamin A into the eluate. Instead of using the dehydrated alfalfa meal as source of the tracer carotene, a heavy band of beta carotene can be produced by mixing ca 100 micrograms of beta carotene (from a solution of crystalline beta carotene in hexane) into the aliquot of the feed extract just prior to passing the solution thru the adsorption column. The beta carotene band (identifiable by its intensity) may be followed as it moves down the column and is slightly eluted.

(e) Under "Procedure" on page 98, line 26, after description of "Colorimetry" add "(See Note 4)" and append the following to the procedure:

NOTE 4: For development of a colorimetric curve, use U.S.P. Vitamin A Reference Standard (U.S.P. Reference Standards, 46 Park Avenue, New York 16, New York). Potency of this standard is 10,000 units of vitamin A per gram. Weigh 0.2 g of the *whole* oil on a small watch glass, wash into a 50 ml volumetric flask with chloroform and make up to volume. Each ml of this solution contains 40 units of vitamin A. From this standard solution make a series of dilutions in chloroform so that 1 ml aliquots when placed in individual colorimeter tubes develop intensities of color which spread over the range of the colorimeter. Suggested concentrations in consecutive tubes might be 5, 10, 20, 30, and 40 units of vitamin A. From the resulting color readings a standard colorimetric curve is established.

(2) The last line "pour extract . . . chromatographic" in Method I for carotene, **40.7,** (page 769), was changed to read as follows and adopted as official:

. . . "pour extract into chromatographic column and use 50 ml or slightly more, if necessary, of acetone-hexane (1+9) to develop chromatogram and wash the visible carotene band thru the adsorbent."

(3) The first action method for nicotinic acid, *This Journal,* **34,** 99–100 (1950), was changed by increasing the strength of sulfuric acid used for

the extraction of nicotinic acid and conversion of nicotinamide to nicotinic acid from 0.25 *N* to 1.0 *N*, and the revised procedure was adopted, first action.

(4) The following microbiological method for pantothenic acid was adopted, first action:

(Applicable only to preparations containing calcium pantothenate or other free forms of pantothenic acid.)

REAGENTS

(a) *Pantothenic acid stock soln. I.*—Dissolve ca 45 mg of U.S.P. Calcium Pantothenate Reference Standard (obtainable from U.S.P. Reference Standards, 46 Park Avenue, New York 16, New York), previously dried and stored in the dark in a desiccator over $P_2O_5$, in ca 500 ml of $H_2O$. Since the reference standard is hygroscopic, weigh in a closed system to prevent absorption of $H_2O$. Add 10 ml of 0.2 *N* acetic acid and 100 ml of 0.2 *N* Na acetate. Finally, add $H_2O$ to make a measured volume that contains 43.47 mmg of calcium pantothenate per ml, and store under toluene at ca 10°.

1.0 ml = 40 mmg of pantothenic acid.

(b) *Pantothenic acid stock soln. II.*—Dil. 100 ml of (a) with ca 400 ml of $H_2O$, add 9 ml of 0.2 *N* acetic acid and 90 ml of 0.2 *N* Na acetate, dil. to 1000 ml, and store under toluene at ca 10°.

1.0 ml = 4.0 mmg of pantothenic acid.

(c) *Standard pantothenic acid soln.*—Dil. 5.0 ml of (b) with $H_2O$ to make 1000 ml. 1.0 ml = 0.02 mmg of pantothenic acid. Prepare fresh standard soln for each assay.

(d) *Acid hydrolyzed casein soln.*—Mix 100 g of vitamin-free casein with 500 ml of constant-boiling HCl (ca 20% HCl) and reflux mixture for 8–12 hours. Remove the HCl from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in $H_2O$, adjust the soln to *p*H 3.5 (±0.1) with 1 *N* NaOH, and add $H_2O$ to make 1000 ml. Add to the soln 20 g of activated charcoal, stir for 1 hour, and filter. Repeat the treatment with activated charcoal. Store under toluene at ca 10°. Filter the soln if a precipitate forms upon storage.

(e) *Cystine-tryptophane soln.*—Suspend 4.0 g of l-cystine and 1.0 g of l-tryptophane (or 2.0 g of d,l-tryptophane) in 700–800 ml of $H_2O$, heat to 70°–80°, and add 20% HCl, dropwise, with stirring, until the solids are dissolved. Cool, and add $H_2O$ to make 1000 ml. Store under toluene at ca 10°.

(f) *Adenine-guanine-uracil soln.*—Dissolve 0.1 g each of adenine sulfate, guanine hydrochloride, and uracil in 5 ml of warm 20% HCl, cool, and add $H_2O$ to make 100 ml. Store under toluene at ca 10°.

(g) *Riboflavin-thiamine hydrochloride-biotin soln.*—Prepare a soln containing, in each ml, 20 mmg of riboflavin, 10 mmg of thiamine hydrochloride, and 0.04 mmg of biotin by dissolving crystalline riboflavin, crystalline thiamine hydrochloride, and crystalline biotin (free acid) in 0.02 *N* acetic acid. Store, protected from light, under toluene at ca 10°.

(h) *p-Aminobenzoic acid-nicotinic acid-pyridoxine hydrochloride soln.*—Prepare a soln in neutral 25% alcohol to contain 10 mmg of *p*-aminobenzoic acid. 50 mmg of nicotinic acid, and 40 mmg of pyridoxine hydrochloride in each ml. Store at ca 10°.

(i) *Salt soln A.*—Dissolve 25 g of $KH_2PO_4$ and 25 g of $K_2HPO_4$ in $H_2O$ to make 500 ml. Add 5 drops of HCl and store under toluene.

(j) *Salt soln B.*—Dissolve 10 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.5 g of $FeSO_4 \cdot 7H_2O$, and 0.5 g of $MnSO_4 \cdot H_2O$ in $H_2O$ to make 500 ml. Add 5 drops of HCl and store under toluene.

(k) *Basal medium stock soln.*—

Acid-hydrolyzed casein soln = 25 ml.
Cystine-tryptophane soln = 25 ml.
Adenine-guanine-uracil soln = 5.0 ml.
Riboflavin-thiamine-biotin soln = 5.0 ml.
*p*-Aminobenzoic acid-nicotinic acid-pyridoxine soln = 5.0 ml.
Salt soln A = 5.0 ml.
Salt soln B = 5.0 ml.
Dextrose anhydrous = 10 g.
Na acetate, anhydrous = 5.0 g.

Dissolve the anhydrous dextrose and Na acetate in the solns previously mixed, and adjust to *p*H 6.8 with NaOH soln. Finally, add $H_2O$ to make 250 ml.

### PREPARATION OF INOCULUM

(a) *Stock culture of Lactobacillus arabinosus 17-5.*[1]—Dissolve 2.0 g of $H_2C$ soluble yeast extract in 100 ml of $H_2O$, add 0.5 g of anhydrous dextrose, 0.5 g of anhydrous Na acetate, and 1.5 g of agar, and heat the mixture, with stirring, on a steam bath until the agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug the tubes with cotton, steriilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and allow tubes to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of Lactobacillus arabinosus 17-5, incubating for 16–24 hours at any selected temp. between 30° and 37° that is held constant to within ±0.5°, and finally store at ca 10°. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 1 week old.

(b) *Culture medium.*—To each of a series of test tubes containing 5 ml of the basal medium stock soln, add 5 ml of $H_2O$ containing 0.2 mmg of pantothenic acid. Plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and cool.

(c) *Inoculum.*—Transfer cells from the stock culture of Lactobacillus arabinosus 17-5 to a sterile tube containing 10 ml of the culture medium. Incubate this culture for 16–24 hours at any selected temp. between 30° and 37° that is held constant to within ±0.5°. The cell suspension so obtained is the inoculum.

### PREPARATION OF SAMPLE SOLUTION

(Throughout all stages of the procedure, keep the soln below *p*H 7.0 in order to prevent loss of pantothenic acid.)

Place a measured quantity of sample in a flask of suitable size and add a volume of $H_2O$ equal in ml to not less than 10 times the dry weight of the sample in g, but the resulting soln must contain not more than 5 mg of pantothenic acid per ml. Adjust the mixture to *p*H 5.65 (±0.05) with either acetic acid soln or Na acetate soln. If the sample is not readily soluble, comminute so that it may be evenly dispersed in the liquid.

Heat the mixture in an autoclave at 121°–123° (1.1–1.2 kg per sq cm) for ca

[1] Obtainable from the American Type Culture Collection, 2029 M Street, N.W., Washington 6, D. C. as Number 8014.

5 min. and cool. If lumping occurs, agitate the mixture until particles are evenly dispersed. Adjust the mixture, with vigorous agitation, to *p*H 6.0–6.5 with NaOH soln, then add HCl soln immediately until no further precipitation occurs (usually ca *p*H 4.0–4.5). Dilute the mixture to a measured volume that contains more than 0.02 mmg of pantothenic acid per ml and filter thru paper known not to adsorb pantothenic acid. In the case of a mixture that is difficult to filter, centrifuging and/or filtering thru sintered glass (using a suitable analytical filter-aid) may often be substituted for, or may precede, filtering thru paper. Take an aliquot of the clear filtrate and check for dissolved protein by adding, dropwise, first HCl soln and, if no precipitate forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further precipitation occurs, add, with vigorous agitation, NaOH soln to *p*H 6.8, dilute the soln to a final measured volume that contains ca 0.02 mmg of pantothenic acid per ml, and if cloudiness occurs, filter again.

(2) If further precipitation occurs, adjust the soln again to the point of maximum precipitation, dilute to a measured volume that contains more than 0.02 mmg of pantothenic acid per ml, and then filter. Take an aliquot of the clear filtrate and proceed as directed under (1).

### DETERMINATION

Prepare standard pantothenic acid tubes as follows: To duplicate test tubes, add 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml, respectively, of the standard pantothenic acid soln. To each tube add 5.0 ml of basal medium stock soln and $H_2O$ to make 10 ml.

Prepare tubes containing the sample soln as follows: To duplicate test tubes add, respectively, 1.0, 2.0, 3.0 and 4.0 ml of the sample soln. To each tube add 5.0 ml of basal medium stock soln and $H_2O$ to make 10 ml.

After mixing, plug the tubes with cotton or cover with caps, and sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm). (Over-heating of assay tubes may produce unsatisfactory results.) Cool, aseptically inoculate each tube with 1 drop of inoculum, and incubate 72 hours at any selected temp. between 30° and 37° that is held constant to within ±0.5°. (Contamination of the assay tubes with any foreign organism invalidates the assay.)

Titrate the contents of each tube with 0.1 *N* NaOH, using bromothymol blue indicator, or to *p*H 6.8 measured electrometrically.

Prepare a standard curve of the pantothenic acid standard titrations by plotting the average of the titration values expressed in ml of 0.1 *N* NaOH for each level of the pantothenic acid standard soln used, against mmg of pantothenic acid contained in the respective tubes. From this standard curve, determine by interpolation the pantothenic acid content of the sample soln in each tube. Discard any values of more than 0.09 or less than 0.01 mmg of pantothenic acid in each tube. Calculate the pantothenic acid content in each ml of sample soln for each tube. Calculate the pantothenic acid content of the sample from the average of the values obtained from not less than 6 of these tubes that do not vary by more than ±10% from the average. If the titration values of less than 6 of these tubes containing the sample soln are within the range of the titration values of the pantothenic acid standard tubes containing 0.01 to 0.09 mmg of pantothenic acid, the data are insufficient to permit calculation of pantothenic acid content of the sample. Titration values exceeding 1.0 ml for the tubes of the standard pantothenic acid soln series containing 0.0 ml of the soln indicate the presence of an excessive quantity of pantothenic acid in the basal medium stock soln and invalidate the assay.

## 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* | |
|---|---|---|
| **2.26** line 2 | 13 | Change "1 g" to "2 g." |
| **2.28** | 14 | Change "2.8016" in numerator of formula to "1.4008." Add ")" after "$NH_4NO_3$." |
| **2.40(b)** line 2 | 17 | Change "2.422" to "2.425." |
| **5.157** | 86 | Change column headed "Wave length (m$\mu$)" to "Wave length (microns)." |
| **12.7** line 1 | 177 | Change "25.6" to "28.5." |
| **12.22** line 1 | 183 | Insert "**(16.17(d))**" after "tube." |
| **12.23** 5th line from bottom | 184 | Insert "petr." before "ether." |
| **13** | 192 | Delete "WHEAT AND" from title "WHEAT AND WHEAT FLOUR." |
| **13.13** | 195 | (line 4) Insert "**(e)**" after "buffer soln." |
| Title above **13.54** | 207 | Insert "WHEAT," before "RYE." |
| Selected references | 226 | Delete entire reference under "(2)"; change "(3)" to "(2)"; delete entire reference under "(4)." |
| **15.27** line 2 | 234 | Insert "(sp. gr. 1.82–1.83 at 20°)" after "$H_2SO_4$." |
| **15.54** line 1 | 246 | Change "5.0" to "50" and insert "add 20 ml alcohol," after "bottle." |
| **16.24(b)** line 2 | 282 | Change "**29.23(a)** or **(b)**" to "**29.23(b)** or **(c).**" |
| **16.33** line 2 | 284 | Change "**18.16**" to "**18.15.**" |
| **18.12(c)** line 5 | 299 | Change "2-naphthalene-3,6-disulfonic acid" to "3-amino-2,7-naphthalenedisulfonic acid." |
| **18.17(b)** line 1, par. 2 | 301 | Change "ca 0.1 ml" to "1.0 ml." |
| line 1, par. 3 | | Change "1 ml" to "ca 0.1 ml." |
| **22** selected references (9) | 358 | Add "**5,** 58 (1921)." |
| **23.21** | 363 | Add at end of paragraph "g creatinin $\times 1.16 =$ g creatin." |
| **27.36(d)** line 6, par. 2 | 465 | Change "When 2 layers sep." to "Let stand until clear." |
| **28.45** line 1 | 485 | Change "**16.20**" to "**16.19(a)** and **16.20.**" |
| **28.46** | 486 | (line 5) Change "top of flask" to "mouth of tube." |
| **28.50** | 487 | In numerator of formula change "$0.9(100-A)$" to "$0.9(100+A)$." |
| **35.67(b)** line 4 | 725 | Insert "l" between "6" and "separator." |
| **39.18** | 761 | Insert at end of paragraph "for ca 0.1 *N* soln." |
| **39.40(a)** line 2 | 765 | Insert "2 hrs" between "dried" and "at 100°." |
| **41.23** | 838 | Column 22/22 corresponding to sp. gr. 0.9808, change ".79" to ".69"; corresponding to sp. gr. 0.9807 change ".67" to ".77"; corresponding to sp. gr. 0.9806 change ".76" to ".86". |

NOTE: The report on "Changes in Methods" should also be consulted.

## REPORT OF REPRESENTATIVE ON BOARD OF GOVERNORS, CROP PROTECTION INSTITUTE

Until recently the direction of the research operations of the Crop Protection Institute were subject to the direction of a Board of Governors, the personnel of which comprised designates from the several national organizations of the sciences that relate to agriculture. This Association has been one of the collaborative organizations. In September of 1951, however, the Crop Protection Institute and the endowed Boyce Thompson Institute entered into an agreement that virtually constitutes a merger of those two organizations. The resultant operative setup prescribes that the Board of Managers shall designate its own personnel and thereby terminates the prior arrangement for Association designations of the personnel of the Board of Governors.

The membership of the Board of Governors has been almost exclusively representative of Experiment Stations, two of the Board members being Station Directors. The function of the Board has been primarily to test experimentally produced insecticides and fungicides and to screen such products through collaborative arrangements with the several Experiment Stations. The activities of the Institute expedited the determination of the values and properties of proposed insecticides and fungicides, and in so doing the Board has proved beneficial to agriculturists and to manufacturers. It is expected that the work of the Institute will continue along established lines and a discontinuance of a representative from A.O.A.C. does not imply disapproval of the change in the method of the Administration of the Crop Protection Institute as a consequence of its intimate connection with the endowed Institute.

W. H. MacIntire

Approved.

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## REPORT OF THE SECRETARY-TREASURER

Henry A. Lepper

The Executive Committee was called to order by President Halvorson at 10:00 A.M. in the West Room of the Shoreham Hotel on Sunday, September 30, 1951. All members were present. The audit of the accounts of the Association as reported by John W. Bisselle and Company was presented and accepted. As was to be expected operating losses as reported for the last two years were not repeated. Those losses resulted in large measure from the financial support given to the *Journal*. The Association had adopted the policy of financing the *Journal* from its general funds in preference to attempting to make it self-supporting by increasing the subscription rate. Although the deficit to be met on the *Journal* was approximately that of the previous year and the expenses

of operating the business office increased, the sale of the Seventh Edition has yielded a substantial net profit in the year's operations. The Executive Committee recommends that no change be made in the subscription price of the *Journal*. The reappointment of William F. Reindollar as chairman of the Committee on Recommendations of Referees is recommended by the Executive Committee. There are no other appointments required on the permanent committees this year.

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, INC.

### BALANCE SHEET—SEPTEMBER 26, 1951

**ASSETS**

| | | |
|---|---|---|
| *Current Assets:* | | |
| Cash, Lincoln National Bank | | $ 23,818.91 |
| Office cash fund | | 71.00 |
| Accounts receivable | | 4,489.88 |
| Accrued interest receivable | | 250.00 |
| Inventories | | 18,888.51 |
| *Total Current Assets* | | $ 47,518.30 |
| *Fixed Assets:* | | |
| Furniture and fixtures | | 992.90 |
| *Investments* | | 51,880.00 |
| *Total Assets* | | $100,391.20 |

**LIABILITIES**

| | | |
|---|---|---|
| *Current Liabilities:* | | |
| Social security deductions | | $ 21.72 |
| Withholding tax collections | | 67.40 |
| *Total Liabilities* | | $ 89.12 |

**SURPLUS**

| | | |
|---|---|---|
| *Balance, September 27, 1950* | $69,207.16 | |
| Add: Net income for the fiscal year ended September 26, 1951 | 31,094.92 | |
| *Balance, September 26, 1951* | | 100,302.08 |
| *Total Liabilities and Surplus* | | $100,391.20 |

The increase in responsibilities and duties of the Secretary arising from the expansion of the business of the Association, as well as the continuing enlargement of its field of activities, leads the Executive Committee to recommend the consideration of the addition of an assistant secretary to the staff of officers for action at the next meeting.

Our Association received an invitation to participate in the Diamond Jubilee celebration of the American Chemical Society in New York. Accordingly an appropriate letter of congratulation was addressed to that Society and presented by Dr. Henry Fishbach on behalf of our Associa-

tion. Our President is in receipt of the following letter from the Chemical Society.

"Dear Mr. Halvorson:

The greeting from the Association of Official Agricultural Chemists to the American Chemical Society on the occasion of our Diamond Jubliee is one of the valued mementos of this event. The celebration has left happy memories in the minds of thousands. In the years to come rereading of the gracious letters from our sister societies, presented at this time, will bring back these recollections.

I was gratified that the association of which you are president could participate in the celebration of our seventy-fifth birthday. The American Chemical Society values the close relations which it has with other scientific and engineering organizations. Many of our problems today are similar or even identical. Anything which brings us into more intimate contact should be considered as a step forward.

It is hoped that in the years ahead our two organizations may continue to progress together for the benefit of the science and country we both serve.

Cordially yours,
N. H. FURMAN
President"

Approved.

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## REPORT OF THE COMMITTEE ON NECROLOGY

JOHN E. BACON

Mr. Bacon, who retired as Chief of the State Bureau of Chemistry on July 16, after 39 years of service with the New Jersey State Department of Health, died on August 27, 1951. Following his graduation from Lehigh University with the degree of chemical engineer, Mr. Bacon was appointed assistant State chemist in 1912. He served as a captain in sanitary engineering units of the U. S. Army in France and Germany during World War I. For several years prior to his retirement, he was a member and secretary of the Board of Examiners of Health Officers and Sanitary Inspectors. In 1944, he was elected president of the Central States Food and Drug Officials.

HENRY R. BOND

The death of Mr. Bond on February 1, 1951, at the age of 48, is recorded with regret. Mr. Bond was born June 26, 1902, and took his schooling at Lewis Institute where he graduated in 1922 with a Bachelor of Science degree in chemistry. For several years after his graduation he held the title of Instructor of Chemistry at his Alma Mater. His work with the Food and Drug Administration began in 1930 as a junior chemist at Chicago. In 1939 he was transferred to Kansas City, where he served as chemist until his death.

HAROLD E. BOYD

The death of Mr. Boyd on January 30, 1950, as the result of an automobile accident, greatly shocked all of his friends. Mr. Boyd was born

June 24, 1912, and received his college training at the Missouri School of Mines. He was appointed an inspector in the Kansas City District of the Food and Drug Administration in 1939. He served at Kansas City until February 1, 1945, when he became Resident Inspector at Omaha, Nebraska. He was vitally interested in the work of the Administration and displayed a great deal of ability in carrying out investigations. Even those who had experienced difficulty with the Administration as a result of violative conditions which Inspector Boyd had uncovered, extended condolences at the time of his death.

### Arthur William Clark

Mr. Clark, Emeritus Professor of Chemistry at Cornell University, died suddenly at his home in Geneva, New York, on October 25, 1950. After his retirement in 1944, Mr. Clark was employed as Analytical Chemist for the U. S. Radiator Corporation and spent several hours each week instructing Experiment Station personnel in the technique of the microscopic examination of animal and poultry feeding stuffs.

Mr. Clark was born August 22, 1879, at Glover, Vermont. Following his graduation from the University of Vermont in 1904 with a Bachelor of Science Degree in Chemistry, he spent a year as Assistant Chemist at the Pennsylvania State College. In 1905 he was appointed to the staff of the Experiment Station at Geneva as Assistant Chemist. In 1915 as Associate Chemist, he was placed in charge of the New York State inspection of fertilizers and feeding stuffs. In 1929 he became Professor of Chemistry at Cornell University, acting as Chief of Chemistry Research.

Professor Clark was instrumental in organizing the State fertilizer and feeding stuffs inspection service and in improving the quality of feeds and fertilizers through this service.

### A. Linton Davidson

The death of Mr. Davidson, editor of the Food and Drug News, Food and Drug Divisions, Department of National Health and Welfare, Canada, is reported with regret. During his 25 years of service, he compiled a history of Dominion legislation, which culminated in the present Food and Drug Act of Canada.

Mr. Davidson was born in Scotland and graduated from the College of the Pharmaceutical Society of the University of London. He did graduate work at King's College, University of London, and passed the examinations of the Royal Institute of Chemistry. He was elected Fellow of this Institute in 1916. In 1925 he entered the service to the Canadian Government and became Dominion Analyst of the Department of Health in 1926. Later he was made Secretary of the Canadian Committee on Pharmaceutical Standards. In recent years he had done a great deal of writing for the Health Department.

HARRY D. GARRETT

The death of Mr. Garrett on January 26, 1949, is recorded with profound regret. Mr. Garrett was born in Minneapolis, graduated from the Agricultural School of the University of Minnesota in 1905, did graduate work at that institution, and received an LL.B from the Minnesota College of Law in 1919. He was employed for 8 years in industry, then entered his life-long career of food-law enforcement. From 1916 to 1918 he was Chief Food Inspector, Minneapolis Health Department. His Federal service began in November 1918 as an inspector with the Food and Drug Administration at Minneapolis. In 1920 he became Chief of the Kansas City Station of the Administration. Following this, he became consecutively, Chief of Cincinnati, Chicago, and Boston Stations. Harry D. Garrett's service was characterized by a complete dedication of his abilities to the work of the U. S. Food and Drug Administration.

FRED H. GRAY

We report with regret the death of Mr. Gray on April 30, 1949. He was born on February 20, 1874, served as an inspector for the Food and Drug Administration in Boston and New York from May 1910 until his retirement in December 1940. He enjoyed his work so completely that after his retirement he took every opportunity to discuss food and drug matters with his old co-workers in Boston.

A. RAY HALL

Mr. Hall died unexpectedly November 5, 1950, at his home in Columbia, Missouri. He was born in Clay County, Missouri, on January 5, 1896. After his graduation from the University of Missouri in 1920, he became a chemist in the Fertilizer Laboratory at the State University. For the past 18 years he had been Feed Chemist for the Missouri State Department of Agriculture at Jefferson City.

CHANNING W. HARRISON

A pioneer in Food and Drug enforcement work, Mr. Harrison died on October 8, 1950, after his retirement as Chief of the Minneapolis Station of the U. S. Food and Drug Administration.

Mr. Harrison reported to the old Bureau of Chemistry in 1903 as a student assistant armed with bachelor's and master's degrees in science from Virginia Polytechnic Institute. One of his early assignments was as analyst on Dr. H. W. Wiley's "Poison Squad" experiments. He had the rare privilege of participating in food work from the days before Federal control, through the full period of the enforcement of the 1906 Act and the early years under the present statute. Mr. Harrison was the first Chemist in Charge of the New Orleans Laboratory, in 1907, when enforcement of the 1906 law began. Early in his career in New Orleans, Mr. Harrison almost precipitated an international situation when he

detained an importation of an adulterated food from a friendly nation. President Theodore Roosevelt demanded of Dr. Wiley that he bring this presumptuous young man to Washington to give an account of himself. Harrison courageously faced the situation and in the end was commended by the President of the United States. He did outstanding scientific work on foods and drugs in Washington, New York, Boston, Baltimore, and finally in Minneapolis as Chief of Station.

IRVING R. HOWLETT

Mr. Howlett was born November 27, 1883, and died July 9, 1951. He received his diploma from the Oshkosh State Teachers College in 1906 and graduated from the University of Wisconsin, majoring in Chemistry, in 1914. He taught for a number of years in the Wisconsin public schools but was forced to give up this work on account of loss of hearing. While at the University, he accepted a job as Chemist with the State Dairy and Food Commission and remained in State service until his retirement in 1949. He was a subscriber to the *Journal of the A.O.A.C.* since its inception, and willed his bound volumes of the *Journal* to the laboratory where he had worked for over 38 years.

GEORGE L. KEENAN

Mr. Keenan died on November 4, 1950. He retired in 1944 after 32 years as microanalyst of food and drugs. During his retirement he continued his interest in the microanalytical field by writing papers and through association with the Science Department at Baldwin-Wallace College in Berea, Ohio. Mr. Keenan was the U. S. Food and Drug Administration's expert on crystallography, the optical properties of substances, and the use of the microscope to detect adulteration. He had prepared many papers in these fields for scientific publication.

GUS J. KUHLMAN

Mr. Kuhlman, formerly Chief Chemist of the Pennsylvania State Department of Agriculture, died on March 9, 1951, at his residence in Harrisburg, following an illness of more than a year. He was born in Nanticoke, Pennsylvania, June 19, 1893, and retired from State service in January, 1950.

Mr. Kuhlman was graduated from the Department of Agricultural Chemistry at the Pennsylvania State College in 1915 and came to the Agricultural Department in 1916 as an assistant food chemist. In January, 1942, he was made Chief Chemist in the laboratory of the Bureau of Foods and Chemistry. While associated with the Department Mr. Kuhlman made thousands of chemical analyses in the enforcement of the State food, feed, fertilizer, and similar laws which were administered under supervision of the Bureau of Foods and Chemistry. His testimony on violations of food laws was highly regarded in Commonwealth prosecutions.

DR. HARRY C. MCLEAN

Dr. McLean of the New Jersey State Agricultural Experiment Station died on September 19, 1949, after a short illness, Dr. McLean was connected with Rutgers University for 36 years until his retirement in 1946 and was well known as author or co-author of scores of scientific papers discussing soil science and spray residue on fruits and vegetables. Dr. McLean also held patents on spray residue removal processes.

WALTER W. SCOFIELD

On March 25, 1951, Mr. Scofield, retired chief of the New Jersey Bureau of Food and Drugs, passed away. He became assistant to the chief of the bureau in 1911 and served as chief from 1918 until his retirement in 1948. Under his leadership, the State developed legislative, regulatory, and educational programs which greatly improved consumer protection against adulteration and misbranding. In 1939, Mr. Scofield worked closely with the legislature which adopted for the State a Food, Drug, and Cosmetic Act with similar requirements to the Federal law. He was one of the founders and a past president of the Philadelphia Conference and of the Central Atlantic States Conference of Food and Drug Officials.

DR. HARVEY SEIL

Dr. Seil recently died in Rutherford, New Jersey, at the age of 68. He received his B.A. and Ph.D. degrees from Columbia University. For several years he was employed by the Food and Drug Administration as a chemist in their New York District.

JONAS CAROL, *Chairman*
HARRY J. FISHER
R. H. CARTER

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## REPORT OF THE COMMITTEE ON NOMINATIONS

Your committee proposes the following nominees and moves their election to the respective offices, as designated:

*President,* HENRY A. LEPPER, Food and Drug Administration, Washington, D. C.

*Vice-President,* H. J. FISHER, Connecticut Agricultural Experiment Station, New Haven, Conn.

*Secretary-Treasurer,* K. L. MILSTEAD, Food and Drug Administration, Washington, D. C.

As additional members of the Executive Committee: E. L. Griffin, U. S. Department of Agriculture, Washington, D. C.; Wm. F. Reindollar, State Department of Health, Baltimore, Md.; K. D. Jacob, U. S. Department of Agriculture, Beltsville, Md.; and H. A. Halvorson, Past

President, Department of Agriculture, Dairy and Food, St. Paul, Minn.

W. H. MACINTIRE, *Chairman*
J. J. T. GRAHAM
R. C. BERRY

Approved.

## REPORT OF THE COMMITTEE ON RESOLUTIONS

*Whereas,* the officers of this Association have faithfully carried out their duties and responsibilities throughout the past year and have thoughtfully planned and efficiently conducted this the 65th Annual Meeting of this organization, therefore be it

*Resolved,* that we express our earnest appreciation to President H. A. Halvorson and Secretary-Treasurer Henry A. Lepper for their faithful service.

*Whereas,* the chief objective of this organization is to promote the development of reliable analytical methods and

*Whereas,* this development depends in no small degree upon the work of the committee on Recommendations of Referees, the members of the several other committees, our Referees, Associate Referees, and collaborative analysts, therefore be it

*Resolved,* that we express our appreciation to these workers for having given of their time and energy to this work.

*Whereas,* we all benefit from the inspiration we receive when someone qualified to evaluate the importance of our efforts speaks of them in commendatory terms, therefore be it

*Resolved,* that we express our thanks and appreciation to Commissioner Charles W. Crawford for appearing before us and addressing us in terms so complimentary to our efforts.

*Whereas,* the success of our work and the acceptance of our methods depends upon the active participation of workers in the many various governmental agencies and commercial institutions in the United States and Canada, 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 attend this meeting.

*Whereas,* the success of this meeting has depended upon adequate assembly rooms and facilities, 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 for the successful conduct of this annual meeting.

L. M. BEACHAM, *Chairman*
J. D. PATTERSON
D. J. MITCHELL

Approved.

## CONTRIBUTED PAPERS

# AN INVESTIGATION OF INSECT AND RODENT CONTAMINATION OF WHEAT AND WHEAT FLOUR

By KENTON L. HARRIS, J. F. NICHOLSON, LILA K. RANDOLPH, and J. L. TRAWICK

(Food and Drug Administration, Federal Security Agency, Washington, D. C.)

## I. INTRODUCTION

Problems of insect infestation and rodent contamination of wheat and of infestation in mills have been recognized for the past several years. The need for a study of the relationship of these factors to insect fragments and rodent hairs in flour prompted a series of conferences between representatives of the milling industry and of the government agencies concerned with the problems. As a result the Food and Drug Administration prepared and issued on January 3, 1950, a draft of a proposed survey of wheat and flour. This preliminary draft was submitted for review to representatives of the industry and of the U. S. Department of Agriculture Production and Marketing Administration, Grain Branch, and the Bureau of Entomology and Plant Quarantine, and, after appropriate revision, was adopted as a cooperative working survey in its final form on April 3, 1950. Mimeographed copies of this program were made available to the industry. The primary objectives of the program were stated as follows:

"1. to determine the relationship of insect infestation in wheat to the fragment count in the flour made from it,
2. to evaluate the effectiveness of mill cleaning equipment and procedures in dealing with infestation and contamination of wheat, and
3. to determine the incidence and level of insect infestation in commercial wheat as received at representative flour mills over a 12-month period. This will include a study of both surface infestation and internal infestation."

"The program will also provide information as to the accuracy of the two methods presently available for estimating the insect infestation in wheat and for predicting the fragment count in the finished flour. It is hoped that the use of these methods on the scale called for in the program will result in reducing the time required for the examination, and indicate such adaptations as might be necessary for use of the methods by non-technical personnel without extensive laboratory facilities."

The following brief summary of the 1950 program was included in the April 3 mimeograph:

"*Mill Program*

Sixteen mills will participate in this phase of the program which is directed toward the first two objectives mentioned.

Each mill selected will be visited at intervals of two weeks during April, May, June, July, August, September and October, and at monthly intervals for the remainder of a calendar year. Investigation to extend over a 12-month period from the time initiated.

Each mill to be checked for sanitary conditions upon each visit.

Samples to be collected as follows during each mill visit: 1. Uncleaned wheat; 2. Same wheat after cleaning and just prior to initial break; 3. Flour made from this wheat."

Samples to be examined as follows:

"All wheat samples to be examined, at least initially and until instructions to the contrary are issued, by both the cracking and flotation method and the Bureau of Entomology staining technique, in addition to visual examination for insects and rodent contamination and laboratory examination for surface insects and rodent hairs.

All flour samples to be examined for insect fragments and rodent hairs by pancreatin digestion and gasoline flotation procedure.

This program will cover not fewer than 16 mills and will yield approximately 300 samples of uncleaned wheat, 300 samples of the corresponding wheat after cleaning, and 300 samples of flour, each containing several subdivisions.

"*Wheat Program*

Collection of samples from approximately 1600 individual carloads or cargoes of wheat as received at flour mills in addition to the wheat sampled under the mill program. Samples to be collected every month at specified mills which are distributed so as to represent the principal types of wheat grown in each of the major areas of production of wheat for milling.

Examination of samples of wheat by methods outlined under the mill program above."

The information called for in the wheat and wheat flour inspection phase of the program is listed below. Column 1 gives the question number on the report form and column 2 the information sought. In addition to the questions listed below a complete report on sanitary conditions in the mill was to be included.

### 1. Inspection

| Question No. | Information |
|---|---|
| | *Sample Number* |
| 1 ..... | Mill Code Number |
| 2 ..... | Location of mill by State |
| 3 ..... | Program (1 = wheat; 2 = mill) |
| 4 ..... | Date of collection or inspection (year, month, day) |
| 5 ..... | Sample taken from: 1 = car; 2 = boat; 3 = other. |
| 6 ..... | Size of lot (in 100's bushels) |
| 7 ..... | Type wheat: 1 = hard red spring; 2 = hard red winter; 3 = soft red winter; 4 = white; 5 = mixed; 6 = other. |
| 8 ..... | State of origin of wheat |
| 9 ..... | Type storage from which shipped to the mill: 1 = farm; 2 = country elevator; 3 = terminal elevator; 4 = direct from harvest; 5 = other. |

### 1. Inspection (continued)

| Question No. | Information |
|---|---|
| 10 | USDA Grade: 1, 2, 3, 4, 5, 6 = sample grade; 7 = not graded; 8 = official grade unknown. |
| 11 | Did grade include weevily notation: 1 = yes; 2 = no; 3 = don't know. |
| 12 | Date of last general fumigation. |
| 13 | Was live infestation noted in conveyance while sampling: 1 = yes; 2 = no. |
| 14 | Was lot homogeneous: 1 = yes; 2 = no. |
| 15 | Visual examination by mill employee: 1 = no insects or rodent pellets; 2 = dead insects; 3 = live insects; 4 = insect damaged kernels; 5 = rodent excreta pellets. |
| 16 | Is wheat regarded as suitable for milling by employee: 1 = yes; 2 = no. |
| 17 | Percentage extraction. |
| 18 | Percentage aggregate flour streams. |
| 19 | Significant changes in cleaning equipment: 1 = yes; 2 = no. |

The information called for on "Part 2—Analytical Work" of the form follows:

### 2. Analytical

| Question No. | Information |
|---|---|
| (Card heading) | Sample Number<br>Mill Code Number<br>Location of Mill by State<br>Program: 1 = wheat; 2 = mill.<br>Analyst |
| 20 | Visual examination 1000 g. uncleaned wheat: 1. Damage noted: 1 = yes; 2 = no. 2. Number insects noted; 3. Number rodent pellets. |
| 21 | Visual examination 1000 g. cleaned wheat: 1. Damage noted: 1 = yes; 2 = no. 2. Number of insects noted; 3. Number of rodent pellets. |
| 22 | 200 grams uncleaned wheat for surface filth: 1. Number of rodent hairs; 2. Number of whole storage insects or equiv.; 3. Number of insect fragments. |
| 23 | 200 grams cleaned wheat for surface filth: 1. Number of rodent hairs; 2. Number of whole storage insects or equiv.; 3. Number of insect fragments. |
| 24 | 200 kernels stain test on uncleaned wheat: 1. No. of kernels with weevil egg punctures; 2. Total no. of weevil egg punctures; 3. No. of kernels with weevil feeding punctures; 4. Total no. of weevil feeding punctures; 5. No. of kernels with insect cutting and/or tunneling; 6. Total no. of insect damaged kernels; 7. Total no. of units of insect damage (sum of 2, 4, 5). |

**2. Analytical** (continued)

| Question No. | Information |
|---|---|
| 25 ..... | 200 kernels stain test cleaned wheat: 1. No. of kernels with weevil egg punctures; 2. Total no. of weevil egg punctures; 3. No. of kernels with weevil feeding punctures; 4. Total no. of weevil feeding punctures; 5. No. of kernels with insect cutting and/or tunneling; 6. Total no. of insect damaged kernels; 7. Total no. of units of insect damage (sum of 2, 4, 5); 8. Weight 100 kernels. |
| 26 ..... | Cracking and flotation 100 grams uncleaned wheat: Total whole insects or equivalent. |
| 27 ..... | Cracking and flotation 100 grams cleaned wheat: Total whole insects or equivalent. |
| 28 ..... | Flour examination: 6. Results calculated to straight flour basis: a. Number rodent hairs; b. Number larvae head capsules; c. Number mandibles; d. Number total insect fragments including heads and mandibles. |

With the cooperation of the mills, samples were taken from carloads of wheat by Food and Drug Administration inspectors by methods based on the probing technique of the U. S. Department of Agriculture. Analyses were made in the Food and Drug Administration District laboratories, and the Washington Laboratory and offices acted as control laboratory and administrative headquarters.

The inspection and analytical results were coded and punched on I.B.M. cards to permit machine sorting, tabulation, and partial calculation of the data. (The use of punch card tabulation not only permitted the rapid and accurate handling of the data, but provided a flexible tool by which the information was rearranged and compared.) A total of 1411 valid samples were taken from individual lots totaling approximately 3,850,000 bushels of wheat.[1] These samples were taken from 109[2] mills chosen at random by a statistical procedure which took into account the mill capacity at each sampling point. Only samples collected from May 1, 1950, through April 30, 1951, are included. Approximately 15 samples collected from non-homogeneous lots or broken in transit or not collected as directed in the program, or those with live infestation, or considered by mill representatives as not suitable for milling, were eliminated from this report.

The mill program investigations at 16 cooperating mills involved sampling of wheat before and after cleaning, sampling the finished flours, and a sanitary inspection of the mill during or just prior to the sampling period. The purpose of these inspections was to check on insect and rodent infes-

[1] According to the U.S.D.A., wheat carryover July 1, 1950 was 427,000,000 bu. Wheat harvested during 1950 was 1,026,755,000 bu.

[2] 130 sampling points were originally selected, but for a variety of reasons only 109 could be used.

tation in the mill to be certain that conditions in the mills were not influencing the fragment count of the flour. Samples collected at mills found to be in an insanitary condition were eliminated. Fifteen samples had to be dropped from the survey because of mill contamination. Mills were chosen with due regard for types of wheat being milled, types of cleaning and milling, and size of mill. A total of 266 valid samples was taken from the 16 mills. In one mill where the estimated bin capacity was found to be in error, so that the timing through the mill was off, four samples could not be used. In another mill inaccuracies in the calculated weights of the flour streams invalidated two flour samples. One additional sample was dropped because of an obvious lack of correlation between the wheat and the flour; the flour contained 95 insect fragments per 50 grams although repeated checks of the wheat did not show the presence of insects.

Further work on the testing of selected survey samples by an X-ray technique[3,4] is now being carried out by Dr. Max Milner of Kansas State College and by the Food and Drug Administration. It is hoped that the results on this work will be published at a later date.

## II. SAMPLING PROCEDURES AND ANALYTICAL METHODS

The procedures used for the collection and analysis of samples were as follows:

### A. Sampling in Mill Survey

1. *Uncleaned wheat.*—Sample falling grain dropping onto or off a conveyor just preceding the first cleaning unit (do not sample grain actually on a belt or conveyor of any type). Take 16 fl. oz. (approx.) portions of grain about every 10 minutes over a 25–30 minute period (four samplings) to yield 64 fl. oz. (2 qts.) of grain. Take a complete cross section of the stream each time.

2. *Cleaned wheat just prior to breaking.*—This sample must be obtained from the same lot of wheat sampled under 1, above. Depending upon the delay involved in the tempering process, several hours or a day or more may elapse between sampling of the uncleaned wheat and sampling of the cleaned wheat. Temper times are different for different mill blends. Care must be taken to insure against error at this point.

It is important to sample the cleaned wheat before it receives any break which produces flour for human consumption. If the mill uses any kind of pre-break which makes a small amount of flour for human consumption, treat such a system as a first break and collect sample before the wheat enters this equipment. If, however, the mill uses an Entoleter, all aspirations from which go into feed, so that the Entoleter is strictly a piece of cleaning equipment, sample *after* this treatment. If the mill does not use any pre-break, the wheat may be sampled just prior to entering one of the first break rolls and may be taken within the roll housing. It is not necessary to sample the streams going to all of the first break rolls. Take 16 fl. oz.

[3] Katz, R., Lee, M. R., and Milner, M. X-Ray Inspection of Wheat, Nondestructive Testing, Fall, 1950.

[4] Milner, M. Recent Developments in Methods for Detecting Internally Infested Wheat, *Milling Production*, January, 1951.

portions at approximately 10 minute intervals until 2 quarts of grain have been collected (4 samplings over a 30 minute period).

If any mills are selected in which a fraction of the wheat does not enter the first break rolls but is diverted to a point further along for breaking, it will be necessary to get a 2 quart sample going into the first break rolls and another 2 quart sample from that portion of the stream which is diverted, and to obtain information as to the percentage of the total represented by each so that the resulting subsamples may be re-combined in the laboratory in such proportions as to represent the original wheat. The laboratory should be given specific instructions as to how to re-combine.

3. *Finished flour streams.*—There will probably be a maximum of four flour streams (aggregated) to be sampled in order to represent the entire flour output from the wheat. Sample the generally recognized flour streams, *e.g.*, straight flour, if any, and patent, first clears, and second clears, even though second clears or corresponding product may be going to feed. Sample after the final aggregation and if possible after re-bolting, but make sure that no blending with flour from other sources (bulk storage tanks, etc.) has occurred. If final re-bolting of one grade of flour is done through more than one sifter, take the sample from the aggregate stream from these sifters. It is desirable to get the flour after the final re-bolting if possible, so that it will correspond as nearly as possible to the product which will be received by the consumer. Sample each aggregated stream separately by collecting 8 oz. portions at approximately 10 minute intervals until 1 quart from each stream has been collected (4 samplings over a 30 minute period). All portions from the same stream may be collected in the same container but each stream must be kept separate from the others. Obtain accurate information as to the percentage of the total flour production (including second clears or corresponding product even though being diverted to feed) represented by each aggregated flour stream sampled to permit calculation of analytical results to the basis of straight flour. Percentage represented by each type of flour should be checked by reference to the mill's weight records and the pertinent figures submitted. Also report the percentage extraction of the wheat represented by the aggregated streams sampled.

The flour samples should represent, as nearly as possible, the identical cleaned wheat sampled. Because of the time lag in the milling process, wait approximately 30 minutes after beginning the cleaned wheat sampling to start the flour sampling, unless after discussion with the miller it appears that a different interval would give a better correlation between wheat and flour. The inspector may adjust this schedule to fit the circumstances in the particular mill.

One and one-half hours should be allowed to elapse after a new mill mix goes "on the mill" to permit sweeping out flour from the previous mix before beginning sampling of cleaned wheat.

Fumigate all samples, wheat and flour, by adding 10 ml of chloroform or carbon tetrachloride to a piece of clean absorbent cotton or blotter paper in the top of the container. Immediately close the containers, and tape top on with Scotch tape to insure proper sealing. Pack in an upright position so that the gaseous fumigant will penetrate downward.

## B. Analytical Work in Mill Survey

### *1. Wheat*

a. Preparation of Sample

Render the entire sample homogeneous by use of the Jones Sampler (A.S.T.M. Specification D-271) and then, using the divider, separate out successively two 50–75 gram portions for the staining test, a slightly over 100 gram portion for cracking, a slightly over 200 gram portion for surface flotation, and a 1000 gram portion for visual examination.

b. Examination of Sample

(The uncleaned wheat and the cleaned wheat will be examined by the same procedures.)

(1) *Visual examination.*—Use the 1000 gram portion and examine by spreading a small amount at a time on a large piece of white paper. Report insects, insect-damaged kernels, and/or rodent excreta pellets. If insects are noted, report the number of each type; if rodent excreta pellets are noted, report the number found.

(2) *Flotation procedure for surface infestation and rodent hairs.*—Adjust the slightly over 200 gram portion to 200 grams. Pick out and discard any rodent excreta pellets or pellet fragments. Transfer the sample to a 2 liter trap flask[5] and add sufficient 20% alcohol to cover the wheat. Add 25–35 ml of gasoline, and mix. Fill into the neck with 20% alcohol, trap off, filter through 10XX bolting cloth, repeat the gasoline extraction, filter through the same bolting cloth, and examine at 25–30X for whole insects or equivalent (adults, pupae, larvae, heads, head capsules, cast skins), insect fragments, and rodent hairs.

(3) *Cracking and flotation procedure.*—After cutting out slightly over 100 grams with the Jones sampler, weigh out 100 grams. Transfer a small amount at a time onto a 5 or 8 inch No. 12 U. S. standard sieve and brush with a stiff-bristle brush to work the surface insects through the sieve as nearly quantitatively as practicable. Grind the screened wheat in a burr or Wiley mill set just fine enough to cut the kernels approximately into eighths, or with no granules any coarser than 2 mm. Transfer all of the cracked wheat to a 2 liter trap flask and brush into the container any residue that may remain in the mill after grinding. Add about 400 ml of 60% alcohol and mix thoroughly. Wash down the sides of the flask with sufficient 60% alcohol to bring the total volume to 700–900 ml and soak 10 minutes. Add 35–50 ml of gasoline, mix thoroughly, and allow to stand 5 minutes. Fill with 60% alcohol, allow to stand 30 minutes and trap off into a beaker. Mix 25–30 ml of gasoline into the trap flask, then 5 ml of 60% alcohol, and after 30 minutes trap off into the same beaker. Filter through a 10XX bolting cloth or if a starchy residue remains add sufficient concentrated HCl to make 1–2% acid, bring to a boil, then filter through a 10XX bolting cloth and examine at 15–30X. Count only whole adults, larvae or pupae, or the heads, head capsules, or cast skins from adults, larvae or pupae.

(4) *Staining procedure for weevil egg-plugs and insect damage.*—Using the two 50–75 gram portions separated as directed above, further reduce each by quartering to approximately 100 kernels, then count out exactly 100 kernels, avoiding any tendency toward personal selection of the kernels to be examined. Examine each 100 kernel portion separately as follows:

Put the 100 kernels into a 100 ml beaker, soak them in warm water for 5 minutes, decant the water and cover the kernels with the dye solution (formula below) for 2–3 minutes. Discard the dye solution and wash the kernels free of excess dye.

[5] For description of Wildman trap flask, see *Methods of Analysis, A.O.A.C.*, 1950, pp. 699 and 704.

Examine each kernel individually at 10X. Confirm all doubtful cases by dissection of the kernel.

Report results on each 100 kernel portion separately as provided on the form.

(The dye solution stains the gelatinous egg caps a deep cherry red. Weevil feeding injury, which will not be covered with the gelatinous cap and which is thereby distinguished from a weevil egg-puncture, the entrance and exit holes of the lesser grain borer, mechanical injury, etc., are stained a lighter color. Insect feeding injuries are round and smooth in outline while mechanical injuries are usually irregular. The capped weevil egg-plugs are round and about 0.2–0.3 mm in diameter.)

To permit calculation of results to a weight basis if this becomes necessary, report the weight of a 100 kernel portion of the cleaned wheat to the nearest 0.01 gram.

Make the fuchsin stain as follows:

| | |
|---|---|
| Acid fuchsin (Color Index 692) | 0.5 g. |
| Glacial acetic acid | 50.0 ml. |
| Distilled water | 950.0 ml. |

Mix the distilled water and the glacial acetic acid, then add the acid fuchsin. Inasmuch as there are 2 types of acid fuchsin which vary in staining quality, the fuchsin to be used for this work (C.I. 692) will be furnished to the laboratories from a single source.

### *2. Flour*

Examine each kind of flour separately. Mix well, by thorough rolling, and quarter down to approximately the desired amount. Weigh 50 grams of the flour into a beaker, add about 300 ml of water and immediately stir into a thin, smooth slurry. Add the filtered extract from 5 grams of pancreatin and mix. Adjust to *p*H 8 with $Na_3PO_4$ soln. Add water to bring the final volume up to 400 ml. Readjust the *p*H after about 15 minutes and again after about 45 minutes. Stir in 3 drops of commercial formaldehyde (U.S.P.) and digest 16–18 hours at 30–37° C. Transfer the digested material to a 2 liter trap flask. Add water to bring the final volume to 700–900 ml. Add 25–35 ml of gasoline and stir for 2 minutes. Add sufficient water to bring the floating gasoline layer up into the neck of the flask so that when the stopper is raised the floating layer and about ¼–½ inch of liquid below the interface can be trapped off and decanted. Allow to stand about 30 minutes with intermittent stirring during this period. Spin the stopper to remove flour that has settled onto it and raise the stopper so as to trap off the gasoline layer. Decant off the floating layer into a beaker, and holding the stopper in place, rinse off the rod and neck of the flask with alcohol and then with water. Collect alcohol risings in a separate receptacle and dilute with water to a concentration of not more than 20% alcohol before adding back to the main body of the trappings. Add 15–25 ml of gasoline to the trap flask and stir in vigorously. Add about 10 ml of water, and allow to stand about 30 minutes with intermittent stirring of the bottom layer. Trap off into the same beaker and again rinse the rod and neck of the flask with alcohol and water in that order. Again collect the alcohol rinse separately and dilute before adding it to the main body of the trappings. Transfer the material in the beaker (both trappings) to a 2 liter trap flask, and add water to bring the floating layer into the neck of the flask. Allow to stand 30 minutes, and stir intermittently by working the stopper through the lower layer and against the sides of the flask. (If there is too much gasoline to permit trapping off in one decantation, trap off most of the gasoline immediately and allow the remainder to stand 30 minutes.) Trap off into a beaker. Filter directly (or, if necessary to dissolve starchy material, acidify to 1–2% HCl, bring to a boil, and then filter) through a C.S. & S. ruled No. 410 filter paper, using suction. Examine at 25–30X to locate insect fragments and rodent hairs. Confirm at higher wide-field magnification if necessary.

In addition to the total insect fragment count, make a separate count of the number of whole or broken mandibles, and the number of larvae head capsules and report separately as provided on the form. Identify the type or types of insect(s) which contributed to the fragment count.

### C. Collection of Samples in Wheat Survey

Samples will be collected only from wheat received at flour mills, prior to or during unloading. If possible, the official grade certificate, or an abstract of pertinent information thereon, should be obtained for each lot of wheat sampled. (The sampling instructions are based on those of the Department of Agriculture Grain Inspectors Manual.) The size of the sample shall be not less than approximately two quarts. (Submit entire sample taken; analyst will mix and divide the sample.)

1. *Sampling of car and truck lots.*—In the case of bulk grain in carload lots, truck or wagon lots, or in any other container in which the grain is of about the same depth as in a carload, sample with a double-tube compartment trier 60 inches long by probing in 5 or more places, well distributed in different parts of the car, truck, or other container. At the discretion of the inspector, as many more probings as may be necessary to provide a representative sample should be taken from the grain in different parts of the lot.

Examine each probing for evidence of uneven loading of the car. Also note any evidence of live insect infestation. After the sample has been examined on the sampling cloth or paper, it may be transferred to Seal-Rite ice cream type containers (waxed fiber) for submittal to the laboratory.

2. *Sampling cargo grain.*—In the case of grain received at the mill in a boat, barge, or other vessel, the sample may be taken from the running stream during unloading, by the use of the pelican spout sampler or a smaller device equivalent in design. Take small portions at regular and frequent intervals over a sufficient period of time to be representative of the lot. Do not take samples from grain moving on a horizontal belt where a cut of a full cross-section of the stream is impracticable.

If it is not practicable to sample during unloading, samples may be taken with a double-tube compartment grain trier of either standard or special length by probing at regularly spaced intervals throughout the entire lot, *provided* that all of the grain is accessible for proper sampling. If the lot of grain is of such depth or stowed in such a manner that representative samples of the entire lot cannot be obtained by this method, the grain should be considered as inaccessible for sampling.

Using the sample obtained as above, record and enter the results of the visual examination by the mill employee responsible for receiving grain, and assist him in the identification of any insects noted.

### D. Examination of Wheat Program Samples

Follow procedure given for uncleaned wheat under "Mill Program" above.

### E. Sampling Schedule

1. *Statistical derivation.*—The problem of sampling wheat offered for milling was approached in accordance with recognized statistical sampling techniques. The mills were chosen for sampling in such a manner that as nearly as possible every carload of wheat shipped to mills in the U. S. had an equal probability of being sampled. With costs and personnel limitations in mind, the number of mills was held to about 100 and the number of samples of wheat to between 1,500 and 2,000.

"A List of Flour Mills," showing daily capacities and locations, compiled and published by the *Northwestern Miller*, was used as a "population" for sampling after eliminating those mills known not to be in operation. Mills having capacities

of 200 sacks or less a day were also eliminated from the list, since they ordinarily engage in little or no interstate commerce and contribute only a very small fraction of the total flour production. Mills on the resulting revised list were divided into 4 size groups: (1) those having a capacity of 10,000 or more sacks a day, (2) 5,000–9,999 sacks a day, (3) 1,000–4,999 sacks a day and (4) 201–999 sacks a day. Within each strata the mills were chosen with probabilities proportionate to size and the number of samples assigned to each strata was proportionate to the total milling capacity of the mills in that strata. All of the mills in group (1) were listed for wheat sampling. In order to designate the mills in the other groups, a cumulative list of the daily capacities of the mills in each group was made, with the mills in the geographical sequence given in the *Northwestern Miller's* "List of Flour Mills." A random number was taken from Fisher's table of "Random Numbers" to give a starting point, and a statistically predetermined sampling interval (16,219 sacks a day for group (2) for example) was successively added, starting with the random number. The points at which this interval fell on the cumulative list of mill capacities then designated the mills to be sampled in that group. This procedure resulted in an initial selection of 17 mills from group (2), 62 mills from group (3), and 15 mills from group (4), which, with the 13 mills in group (1), totaled 107 mills. However, several of these mills had been reported as idle or operating intermittently. Therefore 23 additional mills, chosen by a repetition of the technique outlined above, were added to the original list as revised, to rebuild it to the desired number of active mills and to provide some overage to offset the probable inability to get samples in some instances.

Only one carload or cargo was to be sampled each time, at monthly intervals, at mills in size groups 3 and 4. Two carloads each month were to be sampled at mills in group 2 and at some of the mills in group 1. Three carloads each month were to be sampled at the other mills in group 1. The monthly interval was chosen to give a picture of variation in kind and degree of infestation at different times of the year.

2. *Selecting the car.*—Techniques of conducting sampling programs of this kind require that the selection of units be on a random basis. If several cars are available for sampling, the one or ones to be sampled may be chosen by the following procedure:

Arbitrarily number the cars, say from 1 to 10 if 10 cars are available. Place correspondingly numbered slips of paper (or, preferably, numbered beans, marbles or metal-edged paper tags) in a hat (or bag or other appropriate container) and shuffle. Draw, or have the mill employee draw, a number (or numbers, if more than one car is to be sampled), and sample the car or cars corresponding to the number(s) drawn.

## III. MILL SURVEY

The data could be examined in a number of ways. The approach used in this report has been to consider the condition of the flour as basic and to begin the comparisons from this point of reference. To do this it is necessary to use a standard type of flour such as a straight flour. If the mill was manufacturing a straight flour this was used as reference. If several grades of flour were being produced, they were calculated to the basis of a straight flour. Thus, the calculated straight flour fragment count was ascertained by multiplying the fragment count in 50 grams of each type flour by the percentage of each type produced by the mill and adding the adjusted counts. The main usefulness of this approach lies in the fact that the total flour produced (approximately 70% of the wheat) is the only uniform basis of comparison from mill to mill. Many mills actually combine their

flour streams and so produce a straight flour. Other mills blend some streams and keep others separate and so produce two, three, or even more grades and/or blends.

Certain streams are more heavily loaded with insect fragments than others and any evaluation of flour contamination must be made with this in mind.

### A. Comparison of Methods for Wheat

1. *Correlation coefficient for tests for insect contamination.*—The results of various tests showing the relationships of types of contamination (internal *vs.* external; whole insects *vs.* fragments; floated off *vs.* picked out; or obviously apparent *vs.* internal) will be discussed in subsequent portions

Table 1.—*Correlation of wheat tests for insects to fragments in the flour*

| | NO. OF SAMPLES | CORRELATION COEFFICIENT[1] |
|---|---|---|
| On Uncleaned Wheat | | |
| Flour *vs.* cracking | 266 | 0.90 |
| Flour *vs.* stain | 266 | 0.83 |
| Flour *vs.* surface flotation whole insects | 266 | 0.25 |
| Flour *vs.* surface flotation insect fragments | 266 | −0.08 |
| Flour *vs.* pickout | 266 | 0.13 |
| On Cleaned Wheat | | |
| Flour *vs.* cracking | 266 | 0.92 |
| Flour *vs.* stain | 189[2] | 0.80 |
| Flour *vs.* surface flotation whole insects | 182[2] | 0.23 |
| Flour *vs.* surface flotation insect fragments | 183[2] | 0.00 |
| Flour *vs.* pickout | 211[2] | −0.01 |

[1] 1.00 indicates perfect correlation. 0.00 indicates absolutely no relationship. The 0.92 for cracking test cleaned wheat is very good.
[2] Cleaning in some mills involved so much wheat breakage that this test could not be run.

of this report. The purpose of this section is to establish the method (cracking, stain, flotation for surface insects, flotation for surface insect fragments, or macroscopic pickout) which gives the most reliable estimate of the insects in the wheat that produce insect fragments in the flour. Table 1 gives the respective correlation coefficients of all of these tests and shows that both cracking and stain tests on either the uncleaned or cleaned wheats give fairly reliable estimates of probable insect fragments in the flour. The cracking test is superior in this respect.

The correlation for the cracking test on uncleaned wheat (0.90) shows that even when applied to uncleaned wheat the test is indicative of the insect fragment count which may be expected in the flour. The correlation for cleaned wheat (0.92) shows a similar close relationship. While there is a significant correlation of the stain test to insect fragments in flour it is not as high as for the cracking test. Since the cracking test is a more di-

rect measure of whole insects or equivalent in wheat than the stain test, it was selected as the basic method for judgment of infestation in wheat in subsequent sections of this report.

In this survey there was no appreciable difference in the time required for the two tests as applied to multiple samples. In the laboratories of the Food and Drug Administration insect damage could not be detected by the stain test without use of a microscope.

After the survey was started two tests of grain were suggested. Apt[6] discussed a procedure in which the grain was gelatinized with sodium hydroxide. The internal insects were thus rendered visible and were counted. Milner, Barney, and Shellenberger[7] showed that immersion of weevil-infested grain in an aqueous solution of berberine sulfate resulted

TABLE 2.—*Correlation of wheat tests for rodent contamination to hair fragments in the flour*

| | NUMBER OF TESTS | CORRELATION COEFFICIENT |
|---|---|---|
| Uncleaned Wheat | | |
| Pickout for pellets | 266 | 0.47 |
| Surface hairs | 266 | 0.01 |
| Cleaned Wheat | | |
| Pickout for pellets | 212[1] | 0.24 |
| Surface hairs | 183[1] | 0.13 |

[1] Cleaning in some mills involved so much wheat breakage that this test could not be run on all samples.

in the selective absorption of the alkaloid by the egg plug which then fluoresces under ultraviolet light. The effectiveness of these tests was compared with the survey methods as additional studies in the course of the survey. G. E. Keppel worked on the sodium hydroxide method of Apt and G. C. Reed made the berberine sulfate comparisons. Neither test was found to be as sensitive as the cracking-flotation or stain tests. Details of these investigations will be published at a later date.

2. *Correlation coefficient for tests for rodent contamination.*—An examination of the data on the 266 mill samples by the two tests for rodent contamination of the wheat (*i.e.*, visual examination for rodent pellets per 1000 grams of wheat, and flotation test for detached rodent hairs per 200 grams of wheat) showed that the macroscopic pickout for pellets was the better (Table 2). Note that correlation of rodent pellets in wheat to rodent hairs in flour is better for the uncleaned than for cleaned wheat.

[6] APT, A. C. "A Method for Detecting Hidden Infestation in Wheat." *Milling Production*, 15, 5, p. 1 May (1950).

[7] MILNER, M., BARNEY, D. L., and SHELLENBERGER, J. A. "Use of Selective Fluorescent Stains to Detect Insect Egg Plugs on Grain Kernels." *Science*, 112, 2922, p. 791, December (1950).

This apparent reversal of what might be expected probably is due to the fact that the removal and breakage of pellets during cleaning lowers the sensitivity of this means of checking the wheat and makes the pickout of pellets more difficult. The low correlation of rodent pellets in wheat to rodent hairs in flour may reflect the low level of rodent pellet contamination in the test portions of wheat, as will be discussed later.

### B. Relationship Between Contamination in the Wheat to Contamination in the Flour

1. *Insect fragments in the flour as related to wheat infestation.*—Certain relationships between wheat and flour contamination can be brought out

Table 3.—*Relationship between insect fragments in the flour to insects in the cleaned and uncleaned wheat*

| Insect frag. 50 g flour: range | Number of samples | Cumulative per cent of total | Insects by cracking 100 g wheat | | | |
|---|---|---|---|---|---|---|
| | | | Uncleaned | | Cleaned | |
| | | | Range | Average | Range | Average |
| 0 | 18 | 6.8 | 0–1 | 0.06 | 0–0 | 0.00 |
| 1–5 | 77 | 35.7 | 0–3 | 0.31 | 0–2 | 0.16 |
| 6–10 | 29 | 46.6 | 0–4 | 0.86 | 0–2 | 0.48 |
| 11–15 | 14 | 51.9 | 0–6 | 1.50 | 0–1 | 0.50 |
| 16–20 | 10 | 55.7 | 1–5 | 2.60 | 0–4 | 1.10 |
| 21–30 | 31 | 67.3 | 0–10 | 3.26 | 0–11 | 2.29 |
| 31–40 | 22 | 75.6 | 1–13 | 4.77 | 0–10 | 3.59 |
| 41–50 | 13 | 80.5 | 2–12 | 6.31 | 2–9 | 4.77 |
| 51–60 | 8 | 83.5 | 3–13 | 7.12 | 3–9 | 4.88 |
| 61–80 | 16 | 89.5 | 4–14 | 8.50 | 3–12 | 5.38 |
| 81–100 | 5 | 91.4 | 2–13 | 9.80 | 5–11 | 6.60 |
| 101–150 | 10 | 95.2 | 4–34 | 10.80 | 3–31 | 8.50 |
| 151 and Over[1] | 13 | 100.0 | 4–78 | 30.77 | 4–58 | 23.38 |
| Total or Average[2] | 266 | | | 4.2 | | 3.0 |

[1] Actual counts: 156, 191, 204, 205, 212, 229, 242, 261, 342, 365, 748, 774, 1022.
[2] Over-all averages; not taken from figures in table.

by relating the insects in wheat and the fragment counts on the corresponding flours. Such a breakdown by range of flour counts is given in Table 3.

In this table the insect fragment counts are listed in column 1 as ranges of increasing magnitude. The number of samples in each range is shown in column 2 with the cumulative percentages of the total in column 3. Column 4 shows the range in number of insects and column 5 the average number of insects found in the uncleaned wheat, while columns 6 and 7

show the same information for the corresponding wheat after cleaning at the mills.

Inspection of this table reveals that over one-third (35.7%) of the total of 266 flour samples contained 5 or less, 55.7% contained 20 or less, and 80.5% contained 50 or less insect fragments per 50 grams of flour. About 9% of the samples contained more than 100 insect fragments per 50 grams. The average insect fragment count for all flour samples was 41.3

TABLE 4.—*Comparison of cracking test to range and averages of corresponding flour counts on all mill samples*

| RESULTS ON CRACKING TEST—WHOLE INSECTS PER 100 GRAMS OF CLEANED WHEAT | NUMBER OF SAMPLES | RESULTS ON THE EXAMINATION OF FLOUR FOR INSECT FRAGMENTS/50 GRAMS | | CUMULATIVE PER CENT OF SAMPLES CONTAINING SPECIFIED NUMBER OF INSECTS OR LESS |
|---|---|---|---|---|
| | | RANGE | AVERAGE | |
| 0 | 116 | 0–34 | 4.8 | 43.6 |
| 1 | 38 | 2–29 | 13.2 | 57.9 |
| 2 | 22 | 2–47 | 24.5 | 66.2 |
| 3 | 24 | 21–104 | 44.3 | 75.2 |
| 4 | 18 | 17–156 | 64.8 | 82.0 |
| 5 | 11 | 36–89 | 62.4 | 86.1 |
| 6 | 9 | 30–91 | 56.9 | 89.5 |
| 7 | 2 | 111–140 | 125.5 | 90.2 |
| 8 | 5 | 49–73 | 58.4 | 92.1 |
| 9 | 2 | 49–57 | 53.0 | 92.9 |
| 10 | 2 | 36–118 | 77.0 | 93.6 |
| 11 | 4 | 25–191 | 101.0 | 95.1 |
| 12 | 2 | 78–204 | 141.0 | 95.9 |
| 13 | 2 | 205–261 | 233.0 | 96.6 |
| 18 | 2 | 229–242 | 235.5 | 97.4 |
| 20 | 1 | — | 342.0 | 97.7 |
| 22 | 1 | — | 212.0 | 98.1 |
| 26 | 1 | — | 365.0 | 98.5 |
| 31 | 1 | — | 114.0 | 98.9 |
| 41 | 1 | — | 748.0 | 99.2 |
| 48 | 1 | — | 1022.0 | 99.6 |
| 58 | 1 | — | 774.0 | 100.0 |

per 50 grams (see Table 5). The average number of insects per 100 grams of the corresponding uncleaned and cleaned wheat increase progressively and in a fairly regular manner with increase of insect fragment counts. The flours with zero count show no insects in the cleaned wheat. However, there are some samples of wheat showing no insects by the cracking test which do have a few insect fragments in the flour. This is probably due to low levels of infestation in the wheat which are not detected by the test. With insect fragment counts of the flour above 40, all of the uncleaned

and cleaned wheats show enough infestation to be measured by the cracking test, *i.e.*, the range no longer reaches down to zero.

The average number of insects per 100 grams for all samples of uncleaned and cleaned wheats was 4.2 and 3.0, respectively, which indicates an over-all reduction in internal infestation by mill cleaning of about one-third. Mill cleaning will be discussed in more detail in a later section.

The general relationship of insect fragment counts of flour to insects in cleaned wheat is also portrayed in a different manner in Table 4. In Table 4 the count of insects per 100 grams of cleaned wheat (column 1) is compared with the range and average insect fragment counts per 50 grams of the related flour, in columns 3 and 4.

As might be expected, the ranges of fragment counts overlap widely at different levels of infestation of the wheat, but once the number of insects reaches 3 there are no flour samples with insect fragment counts in the low 0–5 range. The average fragment counts increase in a fairly regular manner from 0 through 4 insects, but above 4, where the number of samples at the various levels is low, the increase is irregular and general.

Flour prepared from cleaned wheat, represented by the 116 samples containing no insects, showed a maximum insect fragment count of 34 and an average insect fragment count of 4.8. Over one-half (57.9%) of the cleaned wheat samples contained one or less whole insects per 100 grams.

The variation in the number of insects in the uncleaned and cleaned wheat as related to the insect fragment counts of flour is in part due to the fact that the wheat stream may not be homogeneous and does not always closely correspond to the sample of flour. Moreover, the relationship of insects in wheat to insect fragments in flour is complex and may be influenced by a number of factors. Among them are: 1, the type of insects and whether they are alive or dead; 2, the type of wheat; 3, the size and flow of the mill; and 4, the kinds of sieving equipment used.

The difference in the fragmentation of live and dead insects has long been known. Several mill representatives have spoken of this fact as common knowledge in the milling industry. Dead and desiccated insects tend to break up easily. Live insects, especially the larvae, often tend to flatten out and become leather-like and tail off with the germ or shorts. If large live larvae such as cadelles are present, they are found in this leather-like condition in the bran. Since hard wheat is harder to reduce to flour than soft wheat, more insect fragments might be produced from each insect in the hard wheat mill. On the other hand, a great deal of the soft wheat is used to make cake flour, which is reduced to a finer particle size than hard wheat flour. This might tend to produce more insect fragments in the soft wheat flour.

The over-all relationship of insects in wheat to insect fragments in flour, as presented in Tables 3 and 4, is a composite of many variables.

It is interesting and informative to examine this relationship according to the individual mills. This is done in Table 5, in which the mills are listed by code (they will not be further identified) in ascending order of the average fragment count of flour produced (column 4). Data on insects in the correlated uncleaned and cleaned wheats, as determined by the cracking test, is presented in the same manner as in Table 3. The average

TABLE 5.—*Relation of insect fragment count of flour to whole insects in uncleaned and cleaned wheat by mills*

(Summary by range and averages. Arranged in increasing order of average flour count)

| MILL NO. | NUMBER OF SAMPLES | INSECT FRAGMENTS 50 G FLOUR | | CRACKING TEST WHOLE INSECTS—100 G UNCLEANED WHEAT | | CRACKING TEST WHOLE INSECTS—100 G CLEANED WHEAT | | RATIO OF AVERAGE INSECT FRAGMENTS IN FLOUR TO AVERAGE INSECTS IN CLEANED WHEAT (CRACKING TEST) |
|---|---|---|---|---|---|---|---|---|
| | | RANGE | AVERAGE | RANGE | AVERAGE | RANGE | AVERAGE | |
| 323 | 20[1] | 0–5 | 0.8 | 0–1 | 0.1 | 0–1 | 0.1 | |
| 167 | 18 | 0–9 | 2.6 | 0–1 | 0.1 | 0–1 | 0.2 | |
| 615 | 19 | 2–8 | 4.5 | 0–4 | 0.9 | 0–2 | 0.2 | |
| 604 | 19 | 1–12 | 5.2 | 0–2 | 0.4 | 0–1 | 0.2 | |
| 311 | 17 | 0–31 | 5.6 | 0–3 | 0.6 | 0–3 | 0.5 | |
| 143 | 18 | 2–29 | 9.5 | 0–2 | 0.6 | 0–4 | 0.6 | |
| 144 | 13 | 3–35 | 11.4 | 0–5 | 1.3 | 0–4 | 1.2 | 9.3 |
| 403 | 16 | 6–47 | 27.9 | 0–7 | 3.3 | 0–6 | 2.8 | 10.1 |
| 211 | 18 | 1–156 | 32.9 | 0–5 | 2.2 | 0–6 | 1.6 | 21.1 |
| 251 | 13 | 8–62 | 34.5 | 0–7 | 3.0 | 0–5 | 2.2 | 15.4 |
| 111 | 19 | 3–73 | 36.0 | 0–14 | 5.9 | 0–9 | 3.6 | 10.1 |
| 162 | 16 | 4–91 | 40.1 | 0–12 | 3.6 | 0–11 | 2.5 | 16.0 |
| 501 | 15 | 19–110 | 46.5 | 1–10 | 5.9 | 1–4 | 2.7 | 17.4 |
| 538 | 13 | 8–118 | 60.9 | 1–34 | 10.6 | 1–31 | 9.4 | 6.5[2] |
| 805 | 14 | 23–365 | 131.9 | 3–48 | 15.1 | 2–26 | 9.9 | 13.3 |
| 536 | 18 | 22–1022 | 234.4 | 1–78 | 18.2 | 1–58 | 13.7 | 17.1 |
| Total and Averages[3] | 266 | 0–1022 | 41.3 | 0–78 | 4.2 | 0–58 | 3.0 | 13.7 |

[1] There were 3 samples collected in mill 323 during May 1950, May 1, 15, and 31.
[2] This figure includes two complete samplings when the cleaning was undergoing changes. The ratio, not including these first two samples, is 0.8 lower.
[3] The averages are obtained from the over-all totals in the original data and not from the figures in this table which would be weighted by considering each mill equal regardless of the number of samples collected.

insect fragment count of flour produced by the various mills ranges from 0.8 to a high of 234 per 50 grams. It is apparent that the first five mills produce flour with a fragment count that is substantially lower than the remaining eleven. The low average counts for these five mills are attributable to a consistently low level of infestation in the wheat used. The mills are located in areas of the West and North where infestation is not a serious problem in the types of wheat milled. Infestation in the wheat

at the remaining mills varies from slight (mill 143 essentially the same as mill 311 and average fragment count less than double) to extremely heavy, and the average fragment counts of the flour increase in more or less direct proportion to the amount of infestation in the grain.

As will be noted the relationship of insects in wheat to insect fragments in the flour is not the same from mill to mill. For example, mills 538 and 805 with approximately the same average levels (9.4 and 9.9) of insects per 100 grams of cleaned wheat, show average insect fragment counts of flour of about 61 and 132, respectively. This is undoubtedly the result of the operation of one or more of the variables mentioned earlier.

The final column in Table 5 lists the ratio of insect fragments in flour to insects in cleaned wheat. This has been obtained by dividing the average number of insect fragments for the samples of flour by the average

TABLE 6.—*Comparison of rodent hairs in the flour to rodent pellets in the uncleaned and cleaned wheat*

| RODENT HAIRS PER 50 G FLOUR | NO.[1] | PER CENT | RODENT PELLETS IN[2] UNCLEANED WHEAT | | RODENT PELLETS IN[2] CLEANED WHEAT | |
|---|---|---|---|---|---|---|
| | | | RANGE | AVERAGE | RANGE | AVERAGE |
| 0 | 132 | 62.7 | 0–3 | 0.20 | 0–1 | 0.06 |
| 1 | 63 | 29.5 | 0–3 | 0.52 | 0–2 | 0.29 |
| 2 | 13 | 6.3 | 0–4 | 0.62 | 0–1 | 0.08 |
| 3 | 2 | 1.0 | 1–1 | 1.00 | 0–1 | 0.50 |
| 13 | 1 | 0.5 | — | 6.00 | — | 1.00 |
| Total and Avs. (over-all) | 211 | — | 0–6 | 0.35 | 0–2 | 0.14 |

[1] Figures in this table limited to 211 samples on which all analyses were completed. Some cleaned wheats were so cracked that examination was impracticable.
[2] Visual examination of 1000 grams.

number of insects from the corresponding samples of cleaned wheat (the number in column 4 divided by the number in column 8). Since it requires about 70 grams of wheat to produce 50 grams of flour, and 100 grams of wheat is used in the cracking test, the numbers listed in the last column are not a direct expression of the number of insect fragments produced from each whole insect.

It will be noted that ratios are not listed for the first six mills in Table 5. Out of the 111 samples from these six mills only 22 contained any insects (89 were zero) and the average fragment count of the corresponding flours was only 4.6. In the case of these mills it would be meaningless to set up a ratio of insects in the wheat to insect fragments in the flour.

The ratios for the remaining mills vary from 6.5 for mill 538 to 21.1 for mill 211 with an average ratio for all samples (including mills 323, 167, 615, 604, 311, and 143) of 13.7. It is obvious, then, that the relation-

ship of insects in wheat to insect fragments in flour varies appreciably from mill to mill. The multiple and complex factors responsible for such variation are not wholly apparent from the available data and are beyond the scope of the survey. It is recognized that the ratio as calculated for each mill represents only a crude comparison and vastly oversimplifies the relationship. While a statistical evaluation of the data indicates a difference from mill to mill, the data are too scanty to permit a more general conclusion.

2. *Rodent hairs in the flour as related to pellets in the wheat.*—The counts of rodent hairs in 50 gram portions of flour are shown in Table 6. The entire range is from 0 to 13. Approximately 63% of the samples counted 0, and over 90% of the samples are either 0 or 1. Table 7 shows that 77%

TABLE 7.—*Rodent pellets in the cleaned wheat and rodent hairs in the flour according to rodent pellets in the uncleaned wheat*

| RODENT PELLETS IN THE UNCLEANED WHEAT[2] | NO.[1] | PER CENT | RODENT PELLETS IN THE CLEANED WHEAT[2] | | RODENT HAIRS IN THE FLOUR | |
|---|---|---|---|---|---|---|
| | | | RANGE | AVERAGE[3] | RANGE | AVERAGE |
| 0 | 163 | 77.3 | 0–2 | 0.07 | 0–2 | 0.38 |
| 1 | 33 | 15.6 | 0–2 | 0.33 | 0–3 | 0.58 |
| 2 | 7 | 3.3 | 0–1 | 0.29 | 0–1 | 0.71 |
| 3 | 6 | 2.8 | 0–1 | 0.67 | 0–2 | 1.00 |
| 4 | 1 | 0.5 | — | 0.00 | — | 2.00 |
| 6 | 1 | 0.5 | — | 1.00 | — | 13.00 |
| Total, and Avs. (over-all) | 211 | | 0–2 | 0.14 | 0–13 | 0.51 |

[1] Figures in this table limited to 211 samples on which all analyses were completed. Some cleaned wheats were so cracked that examination was impracticable.
[2] Visual examination of 1000 grams.
[3] 186 or 88% show 0 pellets.

of the uncleaned wheat samples and 88% of the cleaned wheat contain no pellets in 1000 grams. On the basis of averages, there is a positive relationship between pellets in the wheat and hairs in the corresponding flour.

### C. EFFECT OF CLEANING

1. *Effect of cleaning on insect contamination.*—As shown in Table 5 the effect of cleaning varies from mill to mill. The over-all reduction is about one-third, but individual mills vary from none to more than 50 per cent reduction.

Table 8 presents a statistical analysis of the effect of cleaning as judged by the cracking test for insects. The mills have been arranged in descending order of average number of insects in the uncleaned wheat. A simple inspection of the data shows that, except for 167 and 323, which show the lowest level of infestation in the uncleaned wheat, all of the mills show a reduction in insects by mill cleaning. The standard error of the differ-

TABLE 8.—*Effect of cleaning as shown by cracking test—internal insects, by mills*

| MILL NO. | NUMBER OF SAMPLES | AVERAGE NO. INSECTS | | EFFECT OF CLEANING | | AV. DIFF. ÷ STD. ERROR OF DIFF., OR "t" VALUE | *SIGNIFICANT **HIGHLY SIGNIFICANT |
|---|---|---|---|---|---|---|---|
| | | UNCLEANED WHEAT | CLEANED WHEAT | AV. UNCLEANED MINUS AV. CLEANED | STD. ERR. OF DIFF. | | |
| 536 | 18 | 18.16 | 13.72 | 4.44 | 1.16 | 3.84 | ** |
| 805 | 14 | 15.07 | 9.93 | 5.14 | 1.69 | 3.04 | ** |
| 538 | 13 | 10.62 | 9.39 | 1.23 | 0.82 | 1.51 | |
| 111 | 19 | 5.95 | 3.58 | 2.37 | 0.47 | 5.08 | ** |
| 501 | 15 | 5.93 | 2.67 | 3.26 | 0.57 | 5.70 | ** |
| 162 | 16 | 3.63 | 2.50 | 1.13 | 0.66 | 1.71 | * |
| 403 | 16 | 3.31 | 2.75 | 0.56 | 0.33 | 1.71 | * |
| 251 | 13 | 3.00 | 2.23 | 0.77 | 0.56 | 1.38 | |
| 211 | 18 | 2.17 | 1.56 | 0.61 | 0.34 | 1.77 | * |
| 144 | 13 | 1.31 | 1.23 | 0.08 | 0.38 | 0.20 | |
| 615 | 19 | 0.95 | 0.16 | 0.79 | 0.29 | 2.70 | ** |
| 311 | 17 | 0.65 | 0.53 | 0.12 | 0.22 | 0.52 | |
| 143 | 18 | 0.61 | 0.55 | 0.06 | 0.19 | 0.30 | |
| 604 | 19 | 0.42 | 0.21 | 0.21 | 0.18 | 1.17 | |
| 167 | 18 | 0.11 | 0.16 | −0.05 | 0.12 | 0.44 | |
| 323 | 20 | 0.05 | 0.05 | 0.00 | 0 | 0 | |
| Total, & Avs. (over-all) | 266 | 4.25 | 3.01 | | | | |

TABLE 9.—*Effect of cleaning on reduction of surface insect fragments by surface flotation—200 g samples, by mill*

| MILL NO. | NUMBER OF SAMPLES[1] | AV. NO. INSECT FRAGMENTS | | EFFECT OF CLEANING | | AV. DIFF. ÷ STD. ERROR OF DIFF., OR "t" VALUE | *SIGNIFICANT **HIGHLY SIGNIFICANT |
|---|---|---|---|---|---|---|---|
| | | UNCLEANED WHEAT | CLEANED WHEAT | AV. UNCLEANED MINUS AV. CLEANED | STD. ERR. OF DIFF. | | |
| 536 | 10 | 4.70 | 0.50 | 4.20 | 2.24 | 1.88 | * |
| 111 | 19 | 11.36 | 2.68 | 8.68 | 3.82 | 2.27 | * |
| 501 | 1 | 12.00 | 3.00 | 9.00 | — | — | |
| 162 | 16 | 4.44 | 2.75 | 1.69 | 0.62 | 2.73 | ** |
| 403 | 1 | 2.00 | 5.00 | −3.00 | — | — | |
| 251 | 12 | 4.17 | 1.75 | 2.42 | 1.16 | 2.08 | * |
| 211 | 4 | 10.00 | 5.50 | 4.50 | 4.29 | 1.05 | |
| 144 | 13 | 6.62 | 0.39 | 6.23 | 1.03 | 6.07 | ** |
| 615 | 19 | 25.63 | 3.21 | 22.42 | 3.90 | 5.75 | ** |
| 311 | 17 | 5.82 | 0.53 | 5.29 | 0.93 | 5.69 | ** |
| 143 | 13 | 63.23 | 0.23 | 63.00 | 16.75 | 3.76 | ** |
| 604 | 19 | 16.68 | 3.58 | 13.10 | 2.67 | 4.91 | ** |
| 167 | 18 | 11.74 | 1.00 | 10.74 | 2.56 | 4.19 | ** |
| 323 | 20 | 10.55 | 0.10 | 10.45 | 2.87 | 3.64 | ** |
| Total, & Avs. (over-all) | 182 | 15.14 | 1.78 | | | | |

[1] Some samples of cleaned wheat from several of the mills and all samples from mills 538 and 805 were so broken in cleaning that surface testing was largely impossible.

ence is a measure of the consistency with which each mill effected a reduction in the level of insects in the individual lots of wheat by mill cleaning; the lower the value the more consistent the reduction and, conversely, the higher the value the less consistent the reduction. The test of the statistical significance of the reduction ("t" value) is derived by dividing the

TABLE 10.—*Effect of cleaning on reduction of rodent pellets (by visual examination 1000 g. of wheat)*

| MILL NO. | NUMBER OF SAMPLES[1] | AVERAGE NO. RODENT PELLETS | | EFFECT OF CLEANING | | AV. DIFF. ÷ STD. ERROR OF DIFF., OR "t" VALUE | **HIGHLY SIGNIFICANT |
|---|---|---|---|---|---|---|---|
| | | UNCLEANED WHEAT | CLEANED WHEAT | AV. UNCLEANED MINUS AV. CLEANED | STD. ERR. OF DIFF. | | |
| 536 | 9 | 0.55 | 0.11 | 0.44 | 0.34 | 1.31 | |
| 805 | 4 | 0.0 | 0 | 0 | 0 | 0 | |
| 111 | 19 | 0.05 | 0 | 0.05 | 0.05 | 1.00 | |
| 501 | 2 | 0.0 | 0 | 0 | 0 | 0 | |
| 162 | 16 | 0.25 | 0.06 | 0.19 | 0.21 | 0.90 | |
| 403 | 6 | 0.17 | 0 | 0.17 | 0.17 | 1.00 | |
| 251 | 13 | 0.08 | 0 | 0.08 | 0.08 | 1.00 | |
| 211 | 18 | 1.33 | 0.33 | 1.00 | 0.31 | 3.19 | ** |
| 144 | 13 | 0.23 | 0.15 | 0.08 | 0.21 | 0.37 | |
| 615 | 19 | 0.68 | 0.21 | 0.47 | 0.14 | 3.38 | ** |
| 311 | 17 | 0.12 | 0 | 0.12 | 0.08 | 1.47 | |
| 143 | 18 | 0.33 | 0.28 | 0.05 | 0.21 | 0.27 | |
| 604 | 19 | 0.37 | 0.32 | 0.05 | 0.16 | 0.33 | |
| 167 | 18 | 0.32 | 0.21 | 0.11 | 0.24 | 0.44 | |
| 323 | 20 | 0.10 | 0.00 | 0.10 | 0.07 | 1.45 | |
| Total, & Avs. (overall) | 211 | 0.35 | 0.13 | | | | |

[1] Some samples of cleaned wheat from several of the mills and all samples from mill 538 were so broken in cleaning that the examination after cleaning was impracticable.

average difference between insects in uncleaned and cleaned wheat by the standard error of the difference for each mill. As will be seen in the table, a statistically significant reduction occurs primarily at those mills which use wheat with high levels of infestation.

Table 9 indicates the effect of cleaning on surface insect fragments as determined by flotation test on 200 gram wheat samples. As might be expected, the reduction of surface insect fragments by cleaning was significant at more of the mills than was the reduction of internal insects. Although surface fragments constitute but a small proportion of the total insect load, the ability to reduce this load significantly may be of interest. Data on the reduction of surface *insects* (by flotation test) by the individual mills are not presented in the report. For all mills the average of

0.48 for surface insects per 200 grams of uncleaned wheat was reduced to an average of 0.26 for the samples of cleaned wheat.

2. *Effect of cleaning on rodent pellets.*—As shown in Table 10 there was a general reduction in the rodent pellet load from an over-all average of 0.35 before cleaning to 0.13 after cleaning. From the available data, the observed differences can be stated to be statistically significant in only two of the mills. This, in part at least, is due to the fact that the figures are numerically low. In the samples taken from any one lot before and after cleaning, the chances of detecting a significant improvement is quite small. It is a well-known fact that it is easier to separate rat pellets from wheat than to separate mouse pellets. Since most of the pellets in the mill samples were the smaller wheat-size mouse pellets it is to be expected that they would be difficult to remove.

TABLE 11.—*Distribution of wheat samples by type of wheat*

| TYPE OF WHEAT | NUMBER OF SAMPLES | PER CENT OF SAMPLES IN SURVEY | PER CENT, U.S.D.A. ESTIMATED[1] |
|---|---|---|---|
| Soft Red Winter | 177 | 12.78 | 19.87 |
| White | 140 | 10.10 | 10.96 |
| Hard Red Spring | 298 | 21.52 | 18.05 |
| Hard Red Winter | 770 | 55.60 | 51.12 |
| Mixed | 26 | | |
| Total | 1411 | | |

[1] Agricultural Statistics 1950, U. S. Department of Agriculture.

## IV. WHEAT SURVEY

The discussion in part III has dealt mainly with the mill survey. A total of 1411 wheat samples from the 109 mills used as sampling points for the wheat survey were analyzed for insect and rodent contamination by the same methods used on wheat samples collected in the mills. One of the 1411 samples of wheat contained 277 insects per 100 grams. This figure compares with the next high count of 125 and so influences the averages used as a basis for comparison of the various categories that it has not been included in the following calculations. Another sample with 30 rodent pellets per 1000 grams contrasts with the next high count of 19 and similarly distorts the comparative picture on rodent pellet contamination. Appropriate notation has been made in cases where samples are dropped from the tables. The number of wheat samples by type of wheat is presented in Table 11. On a percentage basis the types of wheat sampled in the survey agreed closely with U.S.D.A. estimated production.

This classification by wheat type[8] proved to be one of the most useful

[8] The abbreviations HRS, HRW and SRW are used to designate, respectively, Hard Red Spring Wheat, Hard Red Winter Wheat and Soft Red Winter Wheat.

means by which the 1411 samples could be separated into workable groups. Actually, of course, there are groups within groups, *e.g.*, white wheat of California may show tendencies which are distinct from those found in the white wheat of Michigan or New York. Sufficient samples were taken from two of the types, HRW and HRS, to permit a further breakdown into additional valid categories. In spite of their heterogeneity, the white wheats were considered as a distinct type in dealing with insect and rodent contamination.

The data from the mill survey clearly indicate that the best tests (on wheat) for insect fragments and rodent hairs in flour are the cracking test for internal insects per 100 grams and the pickout test for rodent pellets per 1000 grams. Hence, these two tests were used for following the trends of insect infestation and rodent pellet contamination in the 1411 wheat survey samples. The other tests applied are discussed in a later section.

### A. Geographical Trends

A breakdown of data by States reduces the number of samples to such a small number for each State that many of the differences become statistically insignificant. However, an over-all tabulation shows some diverse tendencies for the various areas.

1. *Insects in the wheat.*—Table 12 presents the range and average levels of insect and rodent contamination of wheat by States arranged in increasing order of the average insect count per 100 grams of wheat. There are so few samples from many of the States that the average results are not indicative of the true levels of contamination. On the average the levels of infestation tend to be lower in the West, Northwest, Intermountain and Northern States, or in areas where the wheat is predominantly HRS and white wheat. Even in this general area, except in California, individual samples may show a high degree of infestation. The level is higher in the HRW area of the Central Plains States and still higher in the Midwestern and Eastern States which produce SRW wheat.

Table 13 shows a breakdown of the same information by wheat type and indicates the same trend. A map showing the general distribution of wheat by wheat type appears as Figure 1.

2. *Rodent pellets in the wheat.* An examination of Tables 12 and 13 shows a tendency toward higher rodent excreta contamination in a belt including the Dakotas, Nebraska, Minnesota, Iowa, Illinois, Indiana, and Ohio.

### B. Seasonal Fluctuations in Contamination

To follow the seasonal variations three methods have been chosen for appraising levels of infestation. The first makes use of the average number of whole insects for the samples collected each month. The average, however, is influenced by extreme values especially when few obser-

TABLE 12.—*Wheat by State of origin*

(Insects and rodent pellets)

| STATE | NUMBER SAMPLES | INSECTS BY CRACKING TEST | | RODENT PELLETS BY VISUAL EXAMINATION | | PREDOMINANT TYPES OF WHEAT[3] |
|---|---|---|---|---|---|---|
| | | RANGE | AVERAGE | RANGE | AVERAGE | |
| California | 19 | 0–0 | 0 | 0–1 | 0.05 | White |
| Wisconsin | 1 | — | 0 | — | 0 | HRW, HRS |
| (Canada) | 1 | — | 0 | — | 0 | — |
| Arizona | 2 | 0–0 | 0 | 0–0 | 0 | White, HRW |
| New Mexico | 1 | — | 0 | — | 0 | HRW |
| Montana | 147 | 0–4 | 0.15 | 0–19 | 0.40 | HRS, HRW |
| Washington | 52[1] | 0–2 | 0.23 | 0–3 | 0.22 | White, HRW |
| Oregon | 8 | 0–1 | 0.25 | 0–0 | 0.0 | White, HRW |
| Utah | 10 | 0–2 | 0.30 | 0–0 | 0.0 | White, HRW |
| North Dakota | 95 | 0–9 | 0.30 | 0–7 | 0.77 | HRS |
| Wyoming | 7 | 0–2 | 0.43 | 0–2 | 0.29 | HRW, HRS |
| Minnesota | 16 | 0–5 | 0.75 | 0–6 | 1.31 | HRW, HRS |
| Idaho | 51 | 0–20 | 1.26 | 0–12 | 0.41 | White, HRW |
| Iowa | 10 | 0–6 | 1.50 | 0–2 | 0.50 | HRW |
| New York | 32 | 0–18 | 1.62 | 0–7 | 0.50 | White |
| South Dakota | 33 | 0–19 | 1.70 | 0–11 | 0.54 | HRS |
| Michigan | 17 | 0–13 | 1.82 | 0–4 | 0.41 | White, SRW |
| Colorado | 41 | 0–18 | 2.68 | 0–3 | 0.20 | HRW, HRS |
| Nebraska | 120 | 0–47 | 2.77 | 0–16 | 0.80 | HRW |
| Ohio | 58 | 0–27 | 3.10 | 0–11 | 0.97 | SRW |
| Texas | 40 | 0–27 | 4.72 | 0–2 | 0.12 | HRW |
| Indiana | 46 | 0–36 | 4.74 | 0–5 | 1.04 | SRW, HRW |
| Kansas | 284 | 0–54 | 4.87 | 0–3 | 0.32 | HRW |
| (Unknown) | 207 | 0–82 | 5.00 | 0–6 | 0.29 | — |
| North Carolina | 3 | 0–16 | 6.67 | 0–0 | 0 | SRW |
| Illinois | 29 | 0–71 | 8.07 | 0–4 | 0.90 | SRW, HRW |
| South Carolina | 2 | 0–18 | 9.00 | 0–0 | 0 | SRW |
| Oklahoma | 58 | 0–125 | 10.00 | 0–5 | 0.29 | HRW |
| Kentucky | 2 | 7–23 | 15.00 | 0–0 | 0 | SRW |
| Missouri | 15 | 0–107 | 15.33 | 0–4 | 0.27 | SRW |
| Virginia | 4[2] | 6–66 | 28.00 | 0–2 | 0.75 | SRW |
| Total, and Avs. (over-all) | 1411 | | 3.51 | | 0.46 | |

[1] One sample with a pellet count of 30, the highest encountered in the survey, was omitted from these calculations.

[2] One sample with an insect count of 277, the highest encountered in the survey, was omitted from these calculations.

[3] From U.S.D.A. Circular 861. Distribution of the varieties and classes of wheat in the U. S. in 1949.

vations are available. The second basis of comparison is the per cent of samples showing any infestation, that is, samples showing 1 or more insects per 100 grams. The third method involves a comparison of the per cent of samples showing more than 3 insects per 100 grams. This point

TABLE 13.—*Wheat by type and State of origin*
(Insects and rodent pellets)

| TYPE WHEAT | STATE OF ORIGIN | NUMBER SAMPLES | INSECTS BY CRACKING TEST ON 100 GRAMS | | RODENT PELLETS FROM 1000 GRAMS | |
|---|---|---|---|---|---|---|
| | | | RANGE | AVERAGE | RANGE | AVERAGE |
| HRS | Mont., Wyo., (Canada) | 91 | 0–2 | 0.09 | 0–19 | 0.46 |
| | Minn., N.D., S.D., Iowa | 136 | 0–19 | 0.68 | 0–11 | 0.78 |
| | Nebr., Kans., Okla. | 5 | 0–2 | 0.40 | 0–0 | 0. |
| | Missouri | 1 | — | 0 | — | 0 |
| | Wisc., N.Y. | 2 | 0–3 | 1.50 | 0–0 | 0 |
| | S.C. | 1[1] | — | 0 | — | 0 |
| | Total—Av. | 236 | 0–19 | 0.45 | 0–19 | 0.63 |
| HRW | Wash., Calif. | 12 | 0–0 | 0 | 0–2 | 0.42 |
| | Mont., Idaho, Wyo., Colo., Utah | 135 | 0–18 | 1.12 | 0–3 | 0.20 |
| | Minn., Iowa, S.D., N.D. | 17 | 0–6 | 1.18 | 0–3 | 0.65 |
| | Nebr., Kans., Okla., Tex. | 496 | 0–125 | 4.99 | 0–16 | 0.42 |
| | Ind., Ill., Mo. | 11 | 0–22 | 4.36 | 0–4 | 0.82 |
| | Total—Av. | 671 | 0–125 | 4.02 | 0–16 | 0.39 |
| SRW | Wash., Calif., N. Mex. | 5 | 0–0 | 0 | 0–0 | 0 |
| | Ohio, Ind., Ill., Mo. | 124 | 0–107 | 6.11 | 0–11 | 0.87 |
| | Va., N.C., S.C., Ky. | 10[2] | 0–66 | 16.89 | 0–2 | 0.30 |
| | Mich. | 2 | 0–0 | 0 | 0–0 | 0 |
| | Total—Av. | 141 | 0–107 | 6.50 | 0–11 | 0.78 |
| White | Calif., Wash., Oreg., Ariz. | 63[3] | 0–2 | 0.23 | 0–3 | 0.12 |
| | Mont., Idaho, Utah | 22 | 0–20 | 1.46 | 0–5 | 0.23 |
| | Ohio, Ind. | 4 | 0–27 | 7.00 | 0–2 | 1.00 |
| | Mich., N.Y. | 44 | 0–18 | 1.66 | 0–7 | 0.43 |
| | Total—Av. | 133 | 0–27 | 1.22 | 0–7 | 0.27 |
| Mixed | Wash. | 2 | 0–0 | 0 | 0–0 | 0 |
| | Mont., Idaho, Colo. | 9 | 0–5 | 1.22 | 0–12 | 1.78 |
| | Minn. | 1 | — | 0 | — | 0 |
| | Kans. | 1 | — | 5.00 | — | 1.00 |
| | Ohio, Ind., Ill. | 8 | 0–17 | 3.5 | 0–4 | 1.62 |
| | Mich. | 2 | 2–5 | 3.5 | 0–4 | 2.00 |
| | Total—Av. | 23 | 0–17 | 2.22 | 0–12 | 1.48 |
| Total, and Averages (over-all) | | 1204[4] | 0–125 | 3.26 | 0–19 | 0.49 |

[1] The identity of the type of wheat in this sample is open to question.
[2] One sample with an insect count of 277 omitted from these calculations.
[3] One sample with a pellet count of 30 omitted from these calculations.
[4] Total samples low because of 207 samples of "unknown" State of origin.

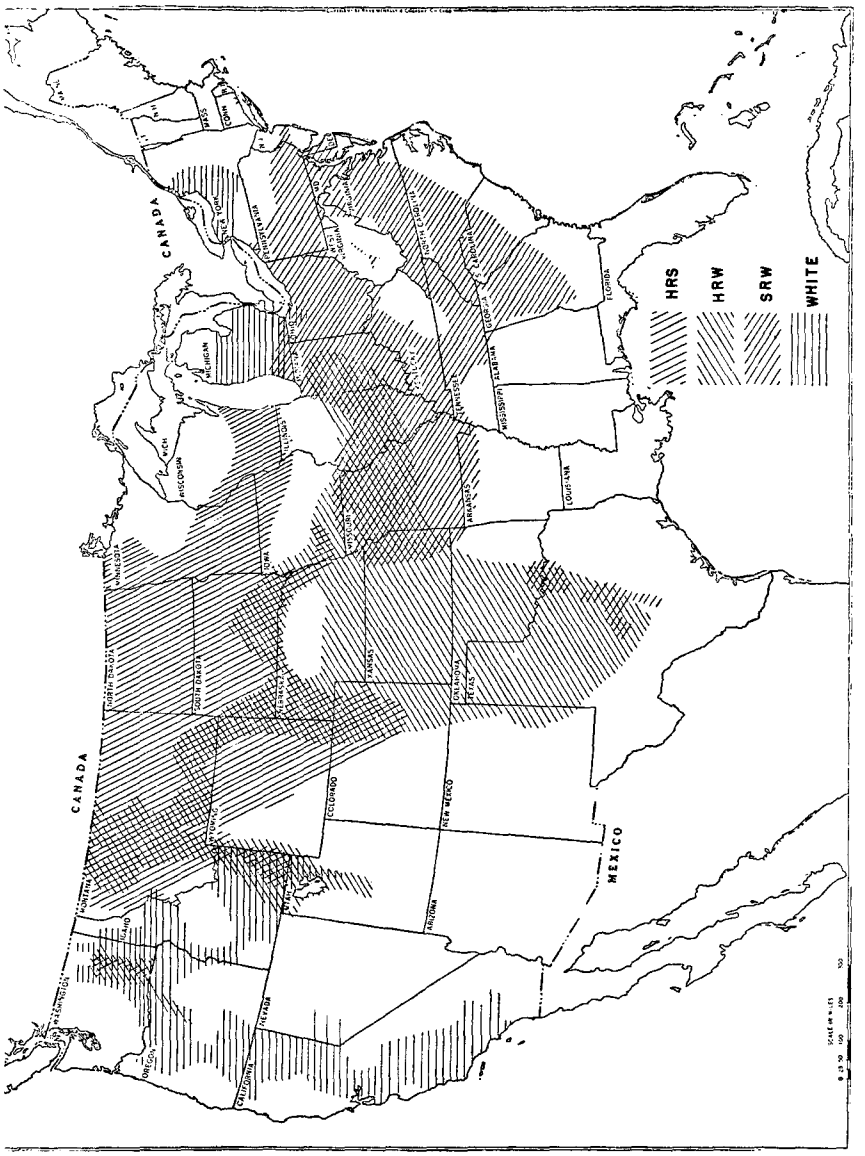



FIG. 1.—Geographical distribution of wheat by types. After U. S. Department of Agriculture Circular #861

was selected only as a means of showing a trend and has no other significance.

1. *Insect contamination in the wheat by month.*—Data on levels of infestation in wheat by month are presented in Table 14 and are plotted in Figure 2. There is a decrease in infestation from May to September with the marketing of new crop wheat, and a rise in October which continues through the winter months. Some very high samples in August

TABLE 14.—*Insect infestation in 1410 wheat samples collected over a twelve month period*

(Cracking test—100 grams)

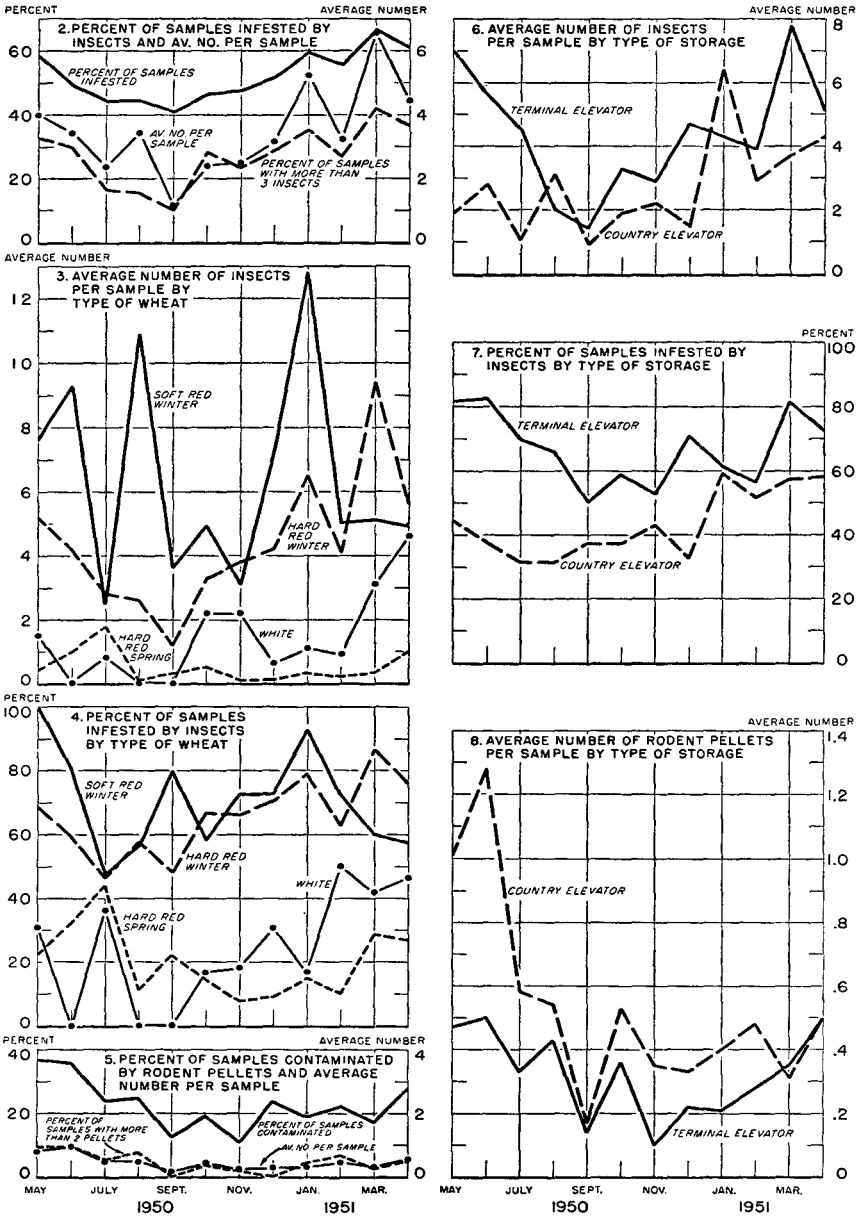
| MONTH | NUMBER OF SAMPLES | AVERAGE INSECTS BY CRACKING | PER CENT SHOWING INFESTATION (1 OR MORE) | PER CENT SHOWING MORE THAN 3 INSECTS |
|---|---|---|---|---|
| May | 128 | 3.99 | 58.59 | 32.81 |
| June | 118 | 3.42 | 49.15 | 29.66 |
| July | 135 | 2.36 | 44.44 | 16.30 |
| Aug. | 130 | 3.45 | 44.62 | 15.38 |
| Sept. | 110[1] | 1.16 | 40.91 | 10.00 |
| Oct. | 110 | 2.40 | 46.36 | 28.18 |
| Nov. | 112 | 2.49 | 47.32 | 23.21 |
| Dec. | 97 | 3.17 | 51.55 | 28.87 |
| Jan. | 116 | 5.23 | 59.48 | 35.34 |
| Feb. | 108 | 3.23 | 55.55 | 26.85 |
| Mar. | 110 | 6.57 | 66.36 | 41.82 |
| Apr. | 136 | 4.45 | 61.03 | 36.76 |

[1] A sample counting 277 insects omitted from these calculations.

raise the average but have little effect on the per cent of samples showing infestation or the per cent of samples showing more than 3 insects. The curve for per cent of samples showing infestation shows the same trends but stays at a more constant level than do the other two curves. All the lines show an upward trend to January, a drop in February, a rise in March and a subsequent drop in April, just at the time the curves would be expected to continue upward if an insect increase associated with warmer weather were the only factor.

In past years the February drop in insect infestation has been reported informally by other investigators and has been attributed to the effect of cold weather, marketing practices, lack of movement of wheat from inaccessible rural areas, and to storage conditions. (The January through April portion of this curve will be discussed in more detail under wheat storage.)

The absence of a more marked decline in infestation with the harvesting of the new crop is explainable by the carry-over of old wheat from year to




FIGS. 2–8. Fluctuations in insect and rodent contamination of wheat over a 12 month period. Insects measured by cracking flotation test per 100 grams. Pellets measured by pickout from 1000 grams.

TABLE 15.—*Infestation in the wheat samples, by type of wheat*

| TYPE OF WHEAT | MONTH | NUMBER OF SAMPLES | AVERAGE | PER CENT SHOWING INFESTATION |
|---|---|---|---|---|
| Hard Red Winter | May | 70 | 5.2 | 68.6 |
| | June | 66 | 4.2 | 59.1 |
| | July | 78 | 2.8 | 46.2 |
| | Aug. | 71 | 2.6 | 57.8 |
| | Sept. | 56 | 1.2 | 48.2 |
| | Oct. | 57 | 3.3 | 66.7 |
| | Nov. | 62 | 3.8 | 66.1 |
| | Dec. | 51 | 4.2 | 70.6 |
| | Jan. | 62 | 6.5 | 79.0 |
| | Feb. | 59 | 4.1 | 62.7 |
| | Mar. | 59 | 9.4 | 86.4 |
| | Apr. | 79 | 5.6 | 76.0 |
| Hard Red Spring | May | 27 | 0.4 | 22.2 |
| | June | 31 | 1.0 | 32.3 |
| | July | 25 | 1.8 | 44.0 |
| | Aug. | 18 | 0.1 | 11.1 |
| | Sept. | 27 | 0.3 | 22.2 |
| | Oct. | 28 | 0.5 | 14.3 |
| | Nov. | 26 | 0.1 | 7.7 |
| | Dec. | 22 | 0.1 | 9.1 |
| | Jan. | 27 | 0.3 | 14.8 |
| | Feb. | 20 | 0.2 | 10.0 |
| | Mar. | 21 | 0.3 | 28.6 |
| | Apr. | 26 | 1.0 | 26.9 |
| Soft Red Winter | May | 15 | 7.6 | 100.0 |
| | June | 10 | 9.3 | 80.0 |
| | July | 19 | 2.5 | 47.4 |
| | Aug. | 23 | 10.9 | 56.5 |
| | Sept. | 15 | 3.6 | 80.0 |
| | Oct. | 12 | 4.9 | 58.3 |
| | Nov. | 11 | 3.1 | 72.7 |
| | Dec. | 11 | 7.3 | 72.7 |
| | Jan. | 14 | 12.8 | 92.9 |
| | Feb. | 18 | 5.0 | 72.2 |
| | Mar. | 15 | 5.1 | 60.0 |
| | Apr. | 14 | 4.9 | 57.2 |
| White | May | 13 | 1.5 | 30.8 |
| | June | 10 | 0.0 | 0.0 |
| | July | 11 | 0.8 | 36.4 |
| | Aug. | 14 | 0.0 | 0.0 |
| | Sept. | 11 | 0.0 | 0.0 |
| | Oct. | 12 | 2.2 | 16.7 |
| | Nov. | 11 | 2.2 | 18.2 |
| | Dec. | 13 | 0.6 | 30.8 |
| | Jan. | 12 | 1.1 | 16.7 |
| | Feb. | 8 | 0.9 | 50.0 |
| | Mar. | 12 | 3.1 | 41.7 |
| | Apr. | 13 | 4.6 | 46.2 |

year, and by the fact that new crop wheat does not reach the market or the mills immediately upon harvest, but is held in farm, country elevator and terminal storage for varying lengths of time. The old crop wheat may continue to flow to the mills or may be blended into the new crop.

Table 15 and Figures 3 and 4 give a breakdown of seasonal variation in infestation by type of wheat. A breakdown by type of wheat tends to separate the samples into groups of common geographical origin.

The winter type wheats remain at higher levels of insect contamination throughout the year. This is shown both by the averages and the per-

TABLE 16.—*Number of rodent pellets in the wheat by visual examination of 1000 grams*

| MONTH | NUMBER OF SAMPLES | AVERAGE NUMBER OF PELLETS | PER CENT OF SAMPLES SHOWING CONTAMINATION | PER CENT OF SAMPLES SHOWING MORE THAN 2 PELLETS/1000 G |
|---|---|---|---|---|
| May | 128 | 0.80 | 36.72 | 9.38 |
| June | 118 | 0.93 | 35.59 | 9.32 |
| July | 135 | 0.47 | 23.70 | 5.18 |
| Aug. | 130 | 0.48 | 24.62 | 7.69 |
| Sept. | 111 | 0.16 | 12.61 | 0.00 |
| Oct. | 109[1] | 0.43 | 19.27 | 3.66 |
| Nov. | 112 | 0.24 | 10.71 | 1.79 |
| Dec. | 97 | 0.28 | 23.71 | 0.00 |
| Jan. | 116 | 0.30 | 18.97 | 4.31 |
| Feb. | 108 | 0.43 | 22.22 | 6.48 |
| Mar. | 110 | 0.31 | 17.27 | 2.73 |
| Apr. | 136 | 0.54 | 27.94 | 5.14 |

[1] A sample counting 30 pellets omitted.

centage with contamination. The wide monthly fluctuations of the average for SRW is, at least in part, due to the small number of samples in this group. The changes in levels of infestation of spring and white wheat lag approximately a month behind the winter wheats.

2. *Rodent contamination in the wheat by month.*—Rodent contamination of the wheat over the 12 month period has been appraised in the same manner as insect infestation. Use is made of the average number of rodent pellets per 1000 grams, the per cent showing pellet contamination, and the per cent showing more than 2 pellets per 1000 grams. Again, the levels of contamination were selected for convenience in presenting the trends, and have no other significance.

Table 16 and Figure 5 show that the curves for the average, the per cent showing any contamination, and the per cent of samples with more than 2 pellets fall from May to September and gradually increase throughout the remainder of the survey period. The marketing of new crop wheat produces a drop in the incidence of rodent pellet contamination. However,

the absence of a more pronounced drop is probably due to the same factors which were discussed in connection with insect infestation.

Since analysis of the data on rodent pellet contamination by wheat type reveals no observable trend, such a breakdown is not presented in this report.

TABLE 17.—*Distribution of samples in relation to the number of insects (100 grams cracking test), including breakdown by type of wheat*

| INSECTS BY CRACKING TEST | NUMBER OF SAMPLES | PER CENT OF SAMPLES | TYPE OF WHEAT | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | HRS | | HRW | | SRW | | WHITE | |
| | | | NO. | PER CENT[1] | NO. | PER CENT[1] | NO. | PER CENT[1] | NO. | PER CENT[1] |
| 0 | 676 | 47.9 | 236 | 79.3 | 268 | 34.8 | 54 | 30.5 | 107 | 76.4 |
| 1 | 173 | 12.3 | 30 | 10.1 | 91 | 11.8 | 31 | 17.5 | 17 | 12.2 |
| 2 | 111 | 7.9 | 13 | 4.4 | 70 | 9.1 | 22 | 12.4 | 4 | 3.0 |
| 3 | 70 | 5.0 | 7 | 2.4 | 56 | 7.3 | 5 | 2.8 | 1 | 0.7 |
| 4 | 71 | 5.0 | 6 | 2.0 | 52 | 6.8 | 10 | 5.6 | 2 | 1.4 |
| 5 | 51 | 3.6 | 1 | 0.3 | 42 | 5.5 | 4 | 2.3 | 1 | 0.7 |
| 6 | 40 | 2.8 | 1 | 0.3 | 32 | 4.2 | 5 | 2.8 | — | — |
| 7 | 32 | 2.3 | — | — | 24 | 3.1 | 7 | 3.9 | 1 | 0.7 |
| 8 | 23 | 1.6 | 1 | 0.3 | 18 | 2.3 | 4 | 2.3 | — | — |
| 9 | 27 | 1.9 | 1 | 0.3 | 24 | 3.1 | 1 | 0.6 | 1 | 0.7 |
| 10 | 22 | 1.6 | 1 | 0.3 | 19 | 2.5 | 2 | 1.1 | — | — |
| 11 | 12 | 0.8 | — | — | 7 | 0.9 | 4 | 2.3 | 1 | 0.7 |
| 12 | 14 | 1.0 | — | — | 11 | 1.4 | 2 | 1.1 | 1 | 0.7 |
| 13 | 10 | 0.7 | — | — | 7 | 0.9 | 2 | 1.1 | 1 | 0.7 |
| 14 | 7 | 0.5 | — | — | 6 | 0.9 | 1 | 0.6 | — | — |
| 15 | 7 | 0.5 | — | — | 6 | 0.8 | 1 | 0.6 | — | — |
| 16– 50 | 54 | 3.8 | 1 | 0.3 | 31 | 4.0 | 18 | 10.2 | 3 | 2.1 |
| 51–100 | 8 | 0.6 | — | — | 5 | 0.6 | 3 | 1.7 | — | — |
| over 100[2] | 3 | 0.2 | — | — | 1 | 0.1 | 2 | 0.6 | — | — |
| Totals | 1411 | 100.0 | 298 | 100.0 | 770 | 100.0 | 178 | 100.0 | 140 | 100.0 |

[1] Per cent of each particular type having given level of infestation.
[2] Actual counts: 277, 125, 107.

### C. GENERAL LEVEL OF INFESTATION IN THE SURVEY SAMPLES

1. *Insect infestation.*—The number and per cent of samples at various levels of infestation are shown in Table 17. Almost 50 per cent of the samples showed no insects. As distributed among the wheat types, HRS (79%) and White (76%) showed the greatest percentages of samples with no insects, while SRW (30%) and HRW (35%) had the lowest percentages of samples with no insects.

2. *Rodent pellet contamination.*—The number and per cent of samples at various levels of contamination are shown in Table 18. Over 76 per cent of the samples showed no pellets as measured by the pickout test on 1000 gram samples.

TABLE 18.—*Distribution of samples in relation to the number of rodent pellets (pickout from 1000 g) including breakdown by type of wheat*

| RODENT PELLETS BY PICKOUT 1000 GRAMS | NUMBER OF SAMPLES | PER CENT OF SAMPLES | BY TYPE OF WHEAT | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | HRS | | HRW | | SRW | | WHITE | |
| | | | NO. | PER CENT[1] | NO. | PER CENT[1] | NO. | PER CENT[1] | NO. | PER CENT[1] |
| 0 | 1084 | 76.9 | 214 | 71.9 | 615 | 79.9 | 120 | 67.4 | 122 | 87.2 |
| 1 | 194 | 13.8 | 56 | 18.8 | 95 | 12.3 | 29 | 16.3 | 9 | 6.4 |
| 2 | 64 | 4.5 | 12 | 4.0 | 35 | 4.5 | 12 | 6.7 | 4 | 2.9 |
| 3 | 33 | 2.3 | 6 | 2.0 | 14 | 1.8 | 10 | 5.6 | 2 | 1.4 |
| 4 | 13 | 0.9 | 4 | 1.3 | 3 | 0.4 | 3 | 1.7 | — | — |
| 5 | 9 | 0.6 | 1 | 0.3 | 3 | 0.4 | 3 | 1.7 | 1 | 0.7 |
| 6 | 3 | 0.2 | 1 | 0.3 | 2 | 0.3 | — | — | — | — |
| 7 | 3 | 0.2 | 2 | 0.7 | — | — | — | — | 1 | 0.7 |
| 8 | — | — | — | — | — | — | — | — | — | — |
| 9 | — | — | — | — | — | — | — | — | — | — |
| 10 | 2 | 0.1 | — | — | 2 | 0.3 | — | — | — | — |
| 11 and over[2] | 6 | 0.5 | 2 | 0.7 | 1 | 0.1 | 1 | 0.6 | 1 | 0.7 |
| Totals | 1411 | 100.0 | 298 | 100.0 | 770 | 100.0 | 178 | 100.0 | 140 | 100.0 |

[1] Per cent of each particular type having given level of infestation.
[2] Actual counts: 11, 11, 12, 16, 19, 30.

TABLE 19.—*Number and per cent of samples with no internal insects or rodent pellets*

| MONTH | TOTAL NUMBER OF SAMPLES | SAMPLES "ZERO" BY BOTH TESTS | |
|---|---|---|---|
| | | NUMBER | PER CENT |
| May 1950 | 128 | 41 | 32 |
| June | 118 | 42 | 36 |
| July | 135 | 56 | 41 |
| August | 130 | 56 | 43 |
| September | 111 | 57 | 51 |
| October | 110 | 51 | 46 |
| November | 112 | 54 | 48 |
| December | 97 | 34 | 35 |
| January 1951 | 116 | 43 | 37 |
| February | 108 | 41 | 38 |
| March | 110 | 33 | 30 |
| April | 136 | 32 | 24 |
| Total | 1411 | 540 | 38 |

In Tables 17 and 18 the number of samples of grain with various levels of insect or rodent contamination are tabulated. An over-all evaluation of contamination involves consideration of both rodent and insect infes-

tation. The monthly and over-all level of wheat showing no insects by the cracking test nor rodent pellets per 1000 grams is given in Table 19. There is an increase in the amount of grain, free from contamination by both tests, associated with the marketing of the new crop. There is a decrease throughout the remainder of the year.

### D. Contamination as Related to Storage

1. *The relationship of insect infestation to type of storage.*—When each survey sample was obtained from the car (or other conveyance) as it arrived at a flour mill, an attempt was made to ascertain, from available records or from the appropriate mill employee, the type of storage from which the wheat had moved to the mill. In some cases this information was not available and in this survey it was not possible to trace shipments back to point of origin. Moreover, the available information revealed nothing as to previous storage of the wheat prior to the storage from which it was actually shipped to the mill. These facts must be taken into account in evaluating the data. Table 20 shows the average for all the samples from the different types of storage.

Table 20.—*Relation of insect infestation to type of storage*

| TYPE OF STORAGE | NUMBER OF SAMPLES | AVERAGE INSECTS PER 100 GRAMS | PER CENT SHOWING INFESTATION |
|---|---|---|---|
| Direct from Harvest | 25 | 0.2 | 12.0 |
| Farm | 41[1] | 6.9 | 31.7 |
| Country Elevator | 782 | 2.8 | 43.9 |
| Terminal Elevator | 542 | 4.4 | 66.8 |
| Other and Unknown | 20 | — | — |
| Total and Avs. (over-all) | 1410 | 3.5 | — |

[1] One sample counting 277 insects omitted.

There were too few samples of wheat direct from harvest, from farm storage, and from "other storage" to permit valid comparisons. There is an obvious difference in insect infestation of wheat from country and terminal elevators. Even though some of the wheat designated "direct from harvest" showed contamination, the degree of contamination was comparatively small. This tends to confirm the belief of authorities on grain infestation that field infestation of ripening wheat by stored grain insects does not normally occur. However, in some areas ripe wheat may become infested if allowed to remain in the field. Samples collected during this survey were not taken in the field during harvest but were sampled upon arrival at the mills. Thus, there was opportunity for them to become contaminated either in temporary farm handling, in farm conveyance, in

TABLE 21.—*Insects in country and terminal elevator samples by month*

| MONTH | COUNTRY ELEVATORS | | | TERMINAL ELEVATORS | | |
|---|---|---|---|---|---|---|
| | NUMBER OF SAMPLES | AVERAGE | PER CENT OF SAMPLES SHOWING INFESTATION | NUMBER OF SAMPLES | AVERAGE | PER CENT OF SAMPLES SHOWING INFESTATION |
| May | 72 | 1.9 | 44.4 | 49 | 7.0 | 81.6 |
| June | 64 | 2.8 | 37.5 | 40 | 5.6 | 82.5 |
| July | 79 | 1.1 | 31.6 | 43 | 4.5 | 69.8 |
| Aug. | 70 | 3.1 | 31.4 | 47 | 2.0 | 66.0 |
| Sept. | 59 | 0.9 | 37.3 | 44 | 1.4 | 50.0 |
| Oct. | 59 | 1.9 | 37.3 | 44 | 3.3 | 59.1 |
| Nov. | 58 | 2.2 | 43.1 | 54 | 2.9 | 52.9 |
| Dec. | 46 | 1.5 | 32.6 | 45 | 4.7 | 71.1 |
| Jan. | 57 | 6.4[1] | 59.6 | 57 | 4.3 | 61.4 |
| Feb. | 64 | 2.9 | 51.6 | 39 | 3.9 | 56.4 |
| Mar. | 61 | 3.7 | 57.4 | 43 | 7.8 | 81.4 |
| Apr. | 93 | 4.3 | 58.1 | 40 | 5.1 | 72.5 |

[1] By dropping two extremely high samples which appear to unduly influence this average it became 3.6, more in line with the yearly trend.

brief elevator handling, or in transit. There is also the possibility that some mixing of old and new crop wheat may have occurred somewhere along the line.

Table 21 and Figures 6 and 7 show the average insect count and per cent of infested samples by month as related to shipments from country or terminal storage. These figures show that the level of infestation for terminal elevator samples, as measured by per cent of samples showing infestation and average insects in the wheat, remains higher throughout the year than for country elevator samples. The infestation levels of wheat from the two sources start to approach each other with the marketing of

TABLE 22.—*Relation of rodent pellet contamination to type of storage*

(Pickout from 1000 g.)

| TYPE OF STORAGE | NUMBER OF SAMPLES | NUMBER OF RODENT PELLETS | |
|---|---|---|---|
| | | RANGE | AVERAGE |
| Direct from Harvest | 25 | 0–5 | 0.20 |
| Farm | 42 | 0–7 | 0.62 |
| Country Elevator | 782 | 0–19 | 0.56 |
| Terminal Elevator | 542 | 0–7 | 0.32 |
| Other and Unknown | 19[1] | 0–4 | 2.00 |
| Total or Avs. (over-all) | 1410 | 0–19 | 0.48 |

[1] One sample counting 30 pellets omitted.

TABLE 23.—*Rodent pellets in country and terminal elevator samples by month*

| MONTH | COUNTRY ELEVATORS | | TERMINAL ELEVATORS | |
|---|---|---|---|---|
| | NUMBER OF SAMPLES | AVERAGE | NUMBER OF SAMPLES | AVERAGE |
| May | 72 | 1.01 | 49 | 0.47 |
| June | 64 | 1.28 | 40 | 0.50 |
| July | 79 | 0.58 | 43 | 0.33 |
| Aug. | 70 | 0.54 | 47 | 0.43 |
| Sept. | 59 | 0.17 | 44 | 0.14 |
| Oct. | 59 | 0.53 | 44 | 0.36 |
| Nov. | 58 | 0.35 | 51 | 0.10 |
| Dec. | 46 | 0.33 | 45 | 0.22 |
| Jan. | 57 | 0.40 | 57 | 0.21 |
| Feb. | 64 | 0.48 | 39 | 0.28 |
| Mar. | 61 | 0.31 | 43 | 0.35 |
| Apr. | 93 | 0.50 | 40 | 0.50 |

the new crop. With fluctuations, the level of infestation in both country and terminal elevator samples then gradually increases through the fall, winter, and early spring months.

2. *Rodent pellets in the wheat as related to type of storage.*—Table 22 presents the breakdown on rodent contamination by type of storage from which the wheat moved to the mills. Table 23 and Figure 8 give a further breakdown by month. Rodent pellet contamination is higher in country elevator than in terminal elevator samples. This is the reverse of the picture shown by internal insect contamination.

### E. RELATIONSHIP BETWEEN THE LEVEL OF INSECT AND RODENT CONTAMINATION AND U.S.D.A. GRADE

There is no direct reference to insect or rodent contamination in the first five U.S.D.A. grades which are based upon quality factors such as moisture and dockage. "Weevily" is not a grain grade but a notation

TABLE 24.—*U.S.D.A. grade in comparison to the number of insects by the cracking test per 100 grams*

| GRADE | NUMBER OF SAMPLES[1] | INSECTS | |
|---|---|---|---|
| | | RANGE | AVERAGE |
| 1 | 801 | 0–125 | 2.57 |
| 2 | 345 | 0–107 | 4.58 |
| 3 | 72 | 0–52 | 5.00 |
| 4 | 14 | 0–78 | 13.64 |
| 5 | 6 | 6–52 | 18.67 |
| Sample | 6 | 0–5 | 0.83 |

[1] The remainder of the samples (167) were "official grade unknown" or "not graded."

applied to a grade. Rodent excreta is a factor only as it renders the wheat "of distinctly low quality" and throws it into "sample" grade.

TABLE 25.—*U.S.D.A. grade in comparison to number of insects in the various types of wheat. (Cracking test per 100 grams)*

| TYPE OF WHEAT | U.S.D.A. GRADE | NUMBER OF SAMPLES | INSECTS PER 100 G | |
|---|---|---|---|---|
| | | | RANGE | AVERAGE |
| HRS | 1 | 243 | 0–19 | 0.57 |
| | 2 | 26 | 0–9 | 0.58 |
| | 3 | 11 | 0–1 | 0.09 |
| | 4 | 2 | 0–1 | 0.50 |
| | 5 | 0 | — | — |
| | Sample | 3 | 0–0 | 0.00 |
| HRW | 1 | 473 | 0–125 | 3.81 |
| | 2 | 196 | 0–82 | 4.92 |
| | 3 | 18 | 0–47 | 8.00 |
| | 4 | 6 | 1–78 | 30.00 |
| | 5 | 5 | 6–52 | 19.80 |
| | Sample | 1 | — | 0.00 |
| SRW | 1 | 21 | 0–18 | 2.33 |
| | 2 | 81 | 0–107 | 7.05 |
| | 3 | 30 | 0–52 | 5.80 |
| | 4 | 5 | 0–6 | 2.00 |
| | 5 | 0 | — | — |
| | Sample | 1 | — | 5.00 |
| White | 1 | 56 | 0–20 | 0.95 |
| | 2 | 33 | 0–4 | 0.30 |
| | 3 | 9 | 0–11 | 2.00 |
| | 4 | 1 | — | 0.00 |
| | 5 | 1 | — | 13.00 |
| | Sample | 0 | — | — |
| Mixed | 1 | 8 | 0–6 | 2.00 |
| | 2 | 9 | 0–6 | 2.11 |
| | 3 | 4 | 0–17 | 5.75 |
| | 4 | 0 | — | — |
| | 5 | 0 | — | — |
| | Sample | 1 | — | 0.00 |

1. *Insect infestation as related to grade.*—Table 24 tabulates the number of samples and the average number of insects in samples by grade. The table shows that as the grade decreases the average level of insect infestation increases except for the designation "sample grade." There were only 6 samples so graded and none of them were down-graded because of weevil damage.

TABLE 26.—*U.S.D.A. grade in comparison to number of rodent pellets in 1000 grams by visual examination*

| GRADE | NUMBER OF SAMPLES | PELLETS | |
|---|---|---|---|
| | | RANGE | AVERAGE |
| 1 | 801 | 0–19 | 0.34 |
| 2 | 344[1] | 0–11 | 0.52 |
| 3 | 72 | 0–16 | 1.17 |
| 4 | 14 | 0–11 | 1.43 |
| 5 | 6 | 0–0 | 0.00 |
| Sample[2] | 6 | 0–3 | 0.50 |

[1] A sample with 30 pellets omitted.
[2] None of these were down-graded because of the presence of rodent pellets.

TABLE 27.—*U.S.D.A. grade in comparison to rodent pellets in the various types of wheat*

| TYPE OF WHEAT | GRADE | NUMBER OF SAMPLES | PELLETS PER 1000 G | |
|---|---|---|---|---|
| | | | RANGE | AVERAGE |
| HRS | 1 | 243 | 0–19 | 0.56 |
| | 2 | 26 | 0–11 | 1.04 |
| | 3 | 11 | 0–4 | 0.55 |
| | 4 | 2 | 0–0 | 0.00 |
| | 5 | 0 | — | — |
| | Sample | 3 | 0–0 | 0.00 |
| HRW | 1 | 473 | 0–6 | 0.26 |
| | 2 | 196 | 0–10 | 0.49 |
| | 3 | 18 | 0–16 | 2.06 |
| | 4 | 6 | 0–4 | 0.67 |
| | 5 | 5 | 0–0 | 0.00 |
| | Sample | 1 | — | 0.00 |
| SRW | 1 | 21 | 0–2 | 0.24 |
| | 2 | 81 | 0–4 | 0.46 |
| | 3 | 30 | 0–5 | 1.00 |
| | 4 | 5 | 0–11 | 3.20 |
| | 5 | 0 | — | — |
| | Sample | 1 | — | 0.00 |
| White | 1 | 56 | 0–3 | 0.11 |
| | 2 | 32 | 0–5 | 0.28[1] |
| | 3 | 9 | 0–1 | 0.22 |
| | 4 | 1 | — | 0.00 |
| | 5 | 1 | — | 0.00 |
| | Sample | 0 | — | 0.00 |
| Mixed | 1 | 8 | 0–2 | 0.63 |
| | 2 | 9 | 0–5 | 1.11 |
| | 3 | 4 | 0–4 | 2.25 |
| | 4 | 0 | — | — |
| | 5 | 0 | — | — |
| | Sample | 1 | — | 3.00 |

[1] A sample with 30 pellets omitted.

The breakdown by type of wheat in Table 25 shows that *the apparent* over-all relationship between grade and insect load reflects the influence of the relatively heavier insect contamination of the HRW, which constitutes more than one-half of the 1411 samples. The average insect load increases with decrease in grade of HRW. Grades 2 and 3 of SRW are higher in internal insects than grade 1. Spring and white wheat show no correlation between grade and insect load and there are many samples with high internal infestation in the better grades.

2. *Rodent contamination as related to grade.*—The data on this subject are presented in Table 26. With decrease in grade through grade 4 there is an increase in the average number of rodent pellets. Grade 5 and sample grade, with six samples in each category, do not follow this trend.

Table 27 gives the breakdown by wheat type. Most of the samples are concentrated in the first two grades. The average number of pellets in grade 2 exceeds those in grade 1 for all wheat types.

3. *Weevily notation as an index of internal insect contamination.*—Out of 1411 samples there were only 12 with weevily notation. Separated by wheat type, eight were HRW (actual counts: 2, 5, 8, 11, 18, 47, 78, 125), three were SRW (actual counts: 4, 11, 107), one was white (count: 0). While weevily notation served to identify some grossly infested lots of wheat, 1228 samples did not bear such a notation and many of these also showed gross internal infestation.

### F. Results of Various Tests on a Comparable Weight Basis

1. *Tests for insects and fragments.*—Table 28 gives the results of averaging the data of the various wheat tests and calculating all tests to a common basis of 1000 grams. On the 1410[9] uncleaned wheat samples the macroscopic pickout averaged 2.1 insects; surface flotation averaged 8.3 insects and 90.2 insect fragments; cracking and flotation for internal insects averaged 37.

More insects are recovered by surface flotation than by macroscopic pickout. With surface insect contamination, a comparison of mill samples shows that insects were reduced from 2.4 to 1.3 and fragments from 68.5 to 8.7. At the same time internal insects in these mill samples were reduced from 42.5 to 30.1. Thus, surface insects in the 1410[9] samples (8.3) are outnumbered by internal (37.0). Surface fragments (90.2) can largely be removed by cleaning. Moreover, they are already fragments which cannot be compared in size with the whole insects recovered by the cracking test. If the cleaning given the mill samples by the 16 mills taking part in that portion of the survey is at all representative of the industry, then mill cleaning does not do a very good job on the internal insects, and they are of primary importance in the problem of flour contamination.

[9] One sample heavily infested with 277 insects per 100 grams not included since it unduly affects the average results.

TABLE 28.—*Comparison of grain tests calculated to same sample weight (1000 grams)*

| TEST | BASIS OF TEST PORTION | | | | CORRECTED TO 1000 GRAMS | | | |
|---|---|---|---|---|---|---|---|---|
| | WHEAT SAMPLES | | MILL SAMPLES | | WHEAT SAMPLES | | MILL SAMPLES | |
| | NUMBER | AVERAGE | NUMBER | AVERAGE | NUMBER | AVERAGE | NUMBER | AVERAGE |
| *Visual exam. 1000 g* | | | | | | | | |
| Insects in uncleaned wheat | 1410 | 2.14 | 211 | 0.24 | 1410 | 2.14 | 211 | 0.24 |
| Insects in cleaned wheat | — | — | 211 | 0.03 | — | — | 211 | 0.03 |
| *Surface flotation 200 g* | | | | | | | | |
| Whole insects, uncleaned | 1410 | 1.66 | 183 | 0.48 | 1410 | 8.30 | 183 | 2.40 |
| Insect fragments, uncleaned | 1410 | 18.04 | 183 | 13.71 | 1410 | 90.20 | 183 | 68.55 |
| Whole insects, cleaned | — | — | 183 | 0.26 | — | — | 183 | 1.31 |
| Insect fragments, cleaned | — | — | 183 | 1.74 | — | — | 183 | 8.69 |
| *Staining 200 kernels*[1] | | | | | | | | |
| Insect damage, uncleaned | 1410 | 1.86 | 189 | 1.68 | 1410 | 309.88 | 189 | 280.39 |
| Insect damage, cleaned | — | — | 189 | 1.43 | — | — | 189 | 238.20 |
| *Cracking test—100 g* | | | | | | | | |
| Insects, uncleaned | 1410 | 3.50 | 267 | 4.25 | 1410 | 37.00 | 267 | 42.51 |
| Insects, cleaned | — | — | 267 | 3.01 | — | — | 267 | 30.08 |

[1] Conversion stain test to 1000 grams is based upon weight 100 kernels wheat =3 grams.

2. *Tests for rodent pellets and hairs.*—Table 29 shows the relationship of the detached rodent hairs to rodent pellets in the wheat on the same

TABLE 29.—*Comparison of grain tests for rodent pellets and hairs calculated to the same weight of sample (1000 grams)*

| TEST | BASIS OF TEST PORTION | | | | CORRECTED TO 1000 G WHEAT | | | |
|---|---|---|---|---|---|---|---|---|
| | WHEAT SAMPLE | | MILL SAMPLE | | WHEAT SAMPLE | | MILL SAMPLE | |
| | NUMBER | AVERAGE | NUMBER | AVERAGE | NUMBER | AVERAGE | NUMBER | AVERAGE |
| *Visual exam.—1000 grams—pellets* | | | | | | | | |
| Uncleaned Wheat | 1410 | 0.48 | 183 | 0.32 | 1410 | 0.48 | 183 | 0.32 |
| Cleaned Wheat | — | — | 183 | 0.14 | — | — | 183 | 0.14 |
| *Surface flotation.—200 grams—hairs* | | | | | | | | |
| Uncleaned Wheat | 1410 | 0.35 | 183 | 0.30 | 1410 | 1.75 | 183 | 1.50 |
| Cleaned Wheat | — | — | 183 | 0.12 | — | — | 183 | 0.60 |

weight basis. As stated earlier (Part III, A, 2) the hairs in the flour relate more closely to pellets rather than to loose hairs in the wheat. In Table 29 the hairs, averaging 1.75 per 1000 grams in the wheat survey, outnumber the pellets, which average 0.48 per 1000 grams. Approximately this same relationship holds for the uncleaned (1.50–0.32) and cleaned (0.60–0.14) wheat of the mill survey. However, it is the experience of this laboratory that one mouse pellet contains several hairs so that actually the pellets in the wheat are carrying more hairs than are contributed by the loose hairs on the wheat. During the survey it was noted that some of the apparent reduction of whole pellets was caused by a breaking of the pellets into small particles that were nearly impossible to pick out from the sample. These pellet fragments would still contribute hairs to the flour.

### G. Field Tests and Observations

One of the purposes of the program was to obtain information on methods for wheat examination that could be used by non-technical personnel. Although the cracking test for internal insects proved to be the most reliable method for determining grain contamination, it requires the use of highly trained personnel and is time consuming. The other laboratory methods currently available are much less sensitive and involve only a minor saving of time. They are better adapted to the testing of individual lots when only one test is to be made. As mentioned earlier, the X-ray technique of Milner (p. 119) is now being investigated. Rodent contamination was checked by picking out the pellets and floating out the hairs.

In addition to these tests, certain procedures used in the survey require no unusual technical equipment or training. These are the visual examination of 1000 gram samples for the presence of insect damaged kernels and for the number of rodent pellets. Either insect damage was seen ("yes") or was not seen ("no"). The rodent pellet count has been discussed earlier. There were three other items to be filled out on the form: (1) Was live infestation noted? (2) Results of visual examination by mill employee for insect damage and rodent pellets. (3) Was the wheat regarded by the mill employee as suitable for milling?

The "suitability for milling" question was included for the purpose of eliminating those samples which, for any reason, would not be used for food purposes by the mill to which it was delivered. Samples unsuitable for milling were dropped from further consideration.

Visual examination by the mill employee was subject to so many vagaries as to render it unsuitable for comparative purposes.

The observation concerning "live infestation noted while sampling" was set up as a rough index of infestation. Its reliability as a means of detecting the presence of insects was sharply limited by the fact that the insects

must be actively alive, and further limited by the fact that the observation was not based upon a specific examination but only upon a condition noted while performing the routine sampling operation. During the survey, inspectors conducting the sampling in railroad cars reported that cold weather prevented an accurate appraisal of this situation. It was apparent that the test became less sensitive as the insects were chilled and became inactive. Over 90 per cent of the samples showed no infestation by this test. In general, the samples showing obvious live infestation also showed more insects by the cracking test (Table 30).

TABLE 30.—*Number of internal insects as related to whether or not live insects were noted during sampling*

(Basis of 12 months)

| TYPE WHEAT | LIVE INFESTATION NOTED | RESULTS BY CRACKING TEST | | |
|---|---|---|---|---|
| | | RANGE | AVERAGE | NUMBER OF SAMPLES |
| HRS | Yes | 0–19 | 4.22 | 9 |
| | No | 0–10 | 0.42 | 289 |
| HRW | Yes | 0–125 | 11.00 | 21 |
| | No | 0–91 | 4.25 | 749 |
| SRW | Yes | 0–107 | 12.00[1] | 27 |
| | No | 0–71 | 5.53 | 150 |
| White | Yes | 0–0 | 0.00 | 3 |
| | No | 0–27 | 1.15 | 137 |
| Mixed | Yes | — | 0.00 | 1 |
| | No | 0–17 | 2.46 | 24 |

[1] One sample containing 277 insects omitted.

1. *Visual examination of 1000 gram sample for evidence of insect infestation.*—The term "hidden infestation" has come into use in the grain and milling industry to refer to what we have in this paper called "internal infestation." The visual examination of a 1000 gram portion was provided for in this program to determine at what level of internal infestation the external signs can routinely be detected with the naked eye and without special laboratory apparatus. It was realized quite early in the program that it would be difficult to standardize this test. Almost all 1000 gram samples of wheat will show some evidence of insect damage if inspected minutely. The problem was to control the test so that it approximated about what might be expected if a person familiar with grain picked up and examined several handfuls. When applied in this manner, actual experience in this laboratory has shown that insect dam-

age was apparent by cursory examination whenever 3 or more insects per 100 grams were found by the cracking test. The difficulty is not in finding external evidence of insect contamination but in judging whether it has reached significant proportions. However, the samples with insect infestation noted in the 1000 grams do run markedly higher by the cracking test. In the case of every wheat type (Table 31) the samples run higher when damage was noted, and the average of 5.38 insects in "damage noted" contrasts with the 0.40 when not noted.

TABLE 31.—*Number of internal insects as related to whether or not insect damage noted in the 1000 gram sample*

| TYPE OF WHEAT | SAMPLES IN WHICH INSECT DAMAGE WAS NOTED IN 1000 GRAMS | | | SAMPLES IN WHICH INSECT DAMAGE WAS NOT NOTED IN 1000 GRAMS | | |
|---|---|---|---|---|---|---|
| | NUMBER OF SAMPLES | INSECTS BY CRACKING TEST 100 GRAMS | | NUMBER OF SAMPLES | INSECTS BY CRACKING TEST 100 GRAMS | |
| | | RANGE | AVERAGE | | RANGE | AVERAGE |
| HRS | 97 | 0–19 | 1.22 | 201 | 0–4 | 0.20 |
| HRW | 602 | 0–125 | 5.54 | 168 | 0–10 | 0.48 |
| SRW | 134[1] | 0–107 | 8.40 | 43 | 0–5 | 0.65 |
| White | 32 | 0–27 | 3.06 | 108 | 0–18 | 0.56 |
| Mixed | 15 | 0–17 | 3.73 | 10 | 0–2 | 0.30 |
| Tot. or Avs. (over-all) | 880 | 0–125 | 5.38 | 530 | 0–18 | 0.40 |

[1] A count of 277 omitted.

Moreover, the tabulation in Table 32 of the 1411 wheat samples by the number of insects cracked from a 100 gram portion indicates that an affirmative answer by the naked eye examination of a 1000 gram sample is a flag that shows that further examination is warranted. It will detect over 90 per cent of the samples which reach a level of 3 or more insects per 100 grams.

2. *Use of a pilot mill as a means of evaluating insect contamination.*—At the suggestion and with the help of one of the commercial mills, a comparison was made of the insect fragment count of flour milled commercially and in one or two Buhler type pilot mills from the same wheat blends. Examination of the commercial and Buhler flours by Food and Drug Administration analysts showed a very close agreement in the insect fragment counts.

3. *Detection of rodent contamination.*—This subject is discussed elsewhere in this report. Of the two tests applied, one was a naked eye pick-out which required as long as 30 minutes when dockage and pellets were high. This is the test used in this report to judge rodent contamination. Many mouse pellets are about the same size and specific gravity as wheat

and the only available method to determine them on a quantitative basis is to pick them out. Such a hand-sorting operation will be expedited if the dockage is examined separately from the dockage-free wheat.

TABLE 32.—*Detection of internal insects by visual examination*

(Cracking test per 100 grams *vs.* presence insect damaged kernels in 1000 grams)

| NUMBER OF INTERNAL INSECTS 100 GRAMS | NUMBER OF SAMPLES | INSECT DAMAGED KERNELS IN 1000 G | | | |
|---|---|---|---|---|---|
| | | DAMAGE NOTED | | DAMAGE NOT NOTED | |
| | | NUMBER | PER CENT | NUMBER | PER CENT |
| 0 | 676 | 245 | 36 | 431 | 64 |
| 1 | 173 | 116 | 67 | 57 | 33 |
| 2 | 111 | 89 | 80 | 22 | 20 |
| 3 | 70 | 64 | 91 | 6 | 9 |
| 4 | 71 | 66 | 93 | 5 | 7 |
| 5 | 51 | 47 | 92 | 4 | 8 |
| 6 | 40 | 39 | 98 | 1 | 2 |
| 7 | 32 | 31 | 97 | 1 | 3 |
| 8 | 23 | 23 | 100 | — | 0 |
| 9 | 27 | 27 | 100 | — | 0 |
| 10 | 22 | 21 | 95 | 1 | 5 |
| 11 | 12 | 12 | 100 | — | 0 |
| 12 | 14 | 13 | 93 | 1 | 7 |
| 13 | 10 | 10 | 100 | — | 0 |
| 14 | 7 | 7 | 100 | — | 0 |
| 15 | 7 | 7 | 100 | — | 0 |
| Over 15 | 65 | 64 | 98 | 1 | 2 |
| Total | 1411 | 881 | — | 530 | — |

## V. SUMMARY AND CONCLUSIONS

1. The insect fragment count of flour (on straight flour basis) is closely related to the number of whole insects or equivalent (insect heads, capsules and cast skins) inside the wheat as measured by the cracking and flotation test for insects. It is somewhat less closely related to insect damage as determined by the fuchsin stain test. There is no correlation between insect fragment count of flour and insects or insect fragments on the surface of wheat. About 44 per cent of the samples of cleaned wheat contained no internal insects in 100 gram samples. Over one-third of the samples of flour contained 5 or fewer insect fragments and about 80 per cent of the samples contained 50 or fewer insect fragments per 50 grams (on a straight flour basis). The average insect fragment count of flour produced by each of 16 mills which were studied varied from a low of 0.8 to a high of 234 per 50 grams. The ratio of insect fragments in flour to whole insects in the wheat from which it was made varies from mill

to mill. The ratio for all mills of fragments in the flour to insects in the cleaned wheat was 13.7 to 1.

2. The rodent pellet pickout on a 1000 gram sample is a practicable test which requires no special training and no laboratory equipment. This test is a better indication of probable flour contamination than is the test for detached rodent hairs in the wheat. The reliability of the pickout test would undoubtedly be increased by the examination of a larger sample. Seventy-seven per cent of the samples of uncleaned wheat and 88 per cent of the samples of cleaned wheat contained no rodent pellets. About 63 per cent of the samples of flour contained no hairs, 30 per cent contained one hair, and 6 per cent contained 2 hairs per 50 grams.

3. The cleaning procedures encountered in the 16 survey mills are relatively ineffective in dealing with internal infestation. Some reduction in the levels of internal infestation was accomplished in practically all cases, and in general the most significant reductions occurred where the levels of infestation were high. However, in those instances, the level of internal infestation remained high in the wheat after cleaning.

Mill cleaning reduced the average number of rodent pellets in wheat at practically all of the mills with an over-all reduction from 0.35 to 0.13 per 1000 grams. A statistically significant reduction in rodent pellets occurred in the two mills with the highest levels of contamination.

4. As measured by the survey methods, 38 per cent of the wheat arriving at flour mills and being milled into flour is free from both internal insect infestation and rodent pellet contamination. During the month of September, when the influence of marketing of new crop wheat is greatest, 51 per cent of the wheat arriving at mills is free of contamination. Since field infestation of wheat is negligible compared to that in storage this high incidence of contamination reflects environmental conditions in the storage and transportation of wheat.

5. Wheat from States in the Western, Northwest, Intermountain and Northern areas, consisting predominantly of white and hard red spring wheat, shows a lower level of insect infestation than from the country as a whole. Wheat from the Central Plains consisting largely of hard red winter wheat shows substantially higher levels of infestation. Soft red winter wheat generally shows high levels of infestation with highest levels in the Midwest and East. There is a tendency toward higher levels of rodent pellets in a belt including the Dakotas, Nebraska, Minnesota, Iowa, Illinois, Indiana, and Ohio.

6. Insect infestation in wheat decreased from a high level at the start of the survey (May) to a low in September. The drop is associated with the harvesting and marketing of new crop wheat. At the lowest level of

infestation in September, 41 per cent of the wheat is infested to some degree. Following the September low there is a general increase, with sharp rises in January and March, and drops in February and April. The winter wheats remain at substantially higher levels of infestation throughout the year than spring or white wheats.

Rodent pellet contamination also decreases with the harvest of the new crop wheat to low points in September and November and increases during the late fall, winter and early spring months.

7. Wheat from terminal elevators shows higher levels of insect infestation and lower levels of rodent pellet contamination than wheat from country elevators.

8. Altho in all U.S.D.A. grades there are instances of high levels of both insect and rodent pellet contamination in individual samples, there is in general a lower average level of contamination in the higher grades.

9. Infestation usually becomes apparent upon visual examination for insect damaged kernels when the level of internal infestation reaches 3 insects per 100 grams. The presence of external insects in the grain, the detection of live infestation in the wheat or in its conveyance while sampling, or the knowledge of prior history, would provide additional clues to the probable presence of internal infestation.

10. The described procedures for both sampling and examining grain appear to have sufficient practicability and reliability to warrant further study and use by the milling and grain handling industries for detection of grain contamination.

## VI. ACKNOWLEDGMENT

To acknowledge completely all of the participants in this work would destroy the anonymity of the various mills. The authors, however, wish to list the inspectors and analysts in their organization who carried the great bulk of the load: J. C. Akers, H. A. Allen, V. F. Balaty, C. R. Baeuerlen, H. P. Bennett, B. F. Campbell, N. A. Carson, W. S. Cox, W. R. Durrenberger, R. T. Elliott, E. W. Greve, W. C. Hill, D. J. Holliday, M. M. Jackson, G. E. Keppel, T. F. Loveridge, F. Y. Mendelsohn, W. H. Munday, R. M. Petrick, G. C. Reed, R. D. Sherman, E. S. Spivak, G. G. Thompson, H. C. Van Dame, and J. T. Welch. The mass of clerical work was handled by M. A. Pelstring. Statistical interpretations and punch card tabulations were by W. Weiss and R. Purdew. The assistance of the Millers' National Federation, Grain and Feed Dealers National Association, and of the U. S. Department of Agriculture is gratefully acknowledged.

# DETERMINATION OF DIPHENYLAMINE IN EXT. D&C YELLOW NO. 1 AND EXT. D&C YELLOW NO. 2

By JOHN E. CLEMENTS and LEE S. HARROW (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

Diphenylamine is an intermediate used in the preparation of Ext. D&C Yellow Nos. 1 and 2. The amount of uncombined diphenylamine in certifiable batches of these colors must not exceed 0.2 per cent.[1] The procedures described below have been developed for the examination of samples of dyes made from this intermediate.

## METHOD

### APPARATUS

Soxhlet extraction apparatus.

A spectrophotometer suitable for measurements at 255, 285, and 315 mμ.

### REAGENT

*Standard diphenylamine soln (10 mg. per liter in 95% alcohol).*

### PROCEDURE

Place 2.5 gm of dye in a cellulose extraction thimble and extract in a Soxhlet extractor with diethyl ether for four hours. Transfer the extract to a 500 ml separatory funnel, wash the extraction flask with two 10 ml portions of ether, and add the washings to the main extract. Shake the combined extract and washings with 30 ml of water made slightly alkaline with NaOH soln. Transfer the ether layer to a beaker and evaporate nearly to dryness on a steam bath. Dissolve the residue in 20 ml of alcohol, transfer to a 100 ml volumetric flask, and dilute to volume with alcohol. Dilute a 10 ml aliquot of this soln to exactly 100 ml with alcohol. Determine the absorbancy of the standard and unknown solns at 255, 285, and 315 mμ with suitable spectrophotometer.

$$\% \text{ Diphenylamine} = \frac{C_s}{25} \times \frac{A_{un}\ 285\ m\mu - \left(\frac{A_{un}\ 225\ m\mu + A_{un}\ 315\ m\mu}{2}\right)}{A_{std}\ 285\ m\mu - \left(\frac{A_{std}\ 225\ m\mu + A_{std}\ 315\ m\mu}{2}\right)}$$

where $C_s$ = concentration of the standard soln expressed in milligrams per liter.
$A_{un}$ = absorbancy of unknown soln.
$A_{std}$ = absorbancy of standard soln.

## DISCUSSION

A sample of diphenylamine (m.p. 52.5–53.5°C. uncor.) recrystallized from hexane was used as a standard. Its absorption curve in alcohol is shown in Figure 1. Solutions of the amine in alcohol follow Beer's law to within ±0.5%.

Weighed samples of purified diphenylamine were added to 2.5 gm

[1] S.R.A., F.D.C. 3, U. S. Food and Drug Adm., Washington, D. C.

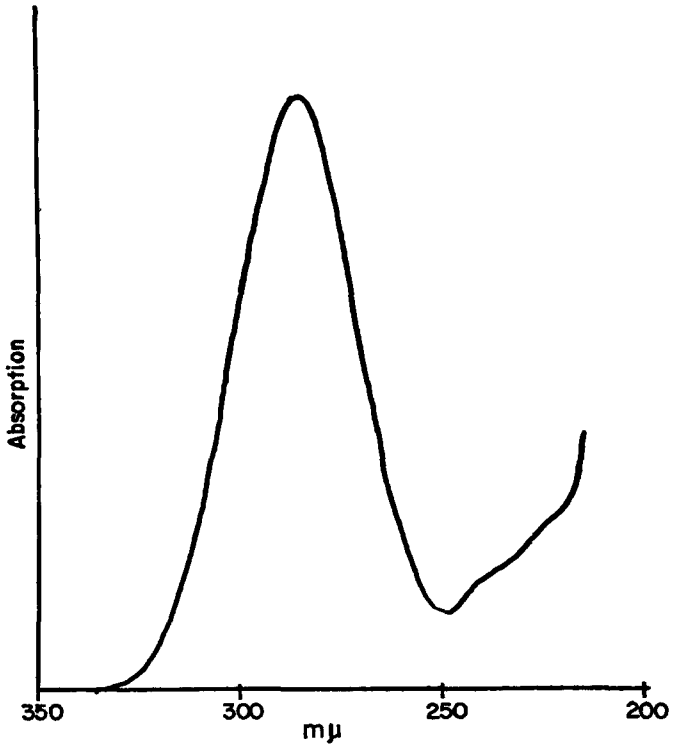



FIG. 1.—Absorption curve of diphenylamine in alcohol (10 mg/liter).

TABLE 1.—*Recovery of diphenylamine from Ext. D&C Yellow No. 1*

| WT. COLOR | DIPHENYLAMINE ADDED | DIPHENYLAMINE FOUND | DIPHENYLAMINE FOUND NET | RECOVERY OF ADDED INTERMEDIATE |
|---|---|---|---|---|
| *g* | *mg* | *mg* | *mg* | *per cent* |
| 2.5 | — | 0.6 | — | — |
| 2.5 | 2.5 | 2.8 | 2.2 | 88 |
| 2.5 | 2.5 | 3.1 | 2.5 | 100 |
| 2.5 | 5.0 | 5.3 | 4.7 | 94 |
| 2.5 | 5.0 | 5.1 | 4.5 | 90 |
| 2.5 | 10.0 | 10.6 | 10.0 | 100 |
| 2.5 | 10.0 | 10.3 | 9.7 | 97 |
| Average | | | | 95 |

TABLE 2.—*Recovery of diphenylamine from Ext. D&C Yellow No. 2*

| WT. COLOR | DIPHENYLAMINE ADDED | DIPHENYLAMINE FOUND | DIPHENYLAMINE FOUND NET | RECOVERY OF ADDED INTERMEDIATE |
|---|---|---|---|---|
| *g* | *mg* | *mg* | *mg* | *per cent* |
| 2.5 | — | 0.5 | — | — |
| 2.5 | 10.0 | 9.8 | 9.3 | 93 |
| 2.5 | 10.0 | 9.0 | 8.5 | 85 |

samples of Ext. D&C Yellow Nos. 1 and 2 and the mixture analyzed by the proposed method; the results are given in Tables 1 and 2.

When the proposed procedure is used, a small amount of color bleeds into the final alcohol solution. A "straight-line background" correction compensates for the presence of a small amount of the color in the final solution. In the equation given, the terms in parenthesis are those needed for the correction.

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## STUDIES ON A MICROBIOLOGICAL METHOD FOR VITAMIN $B_{12}$ ACTIVITY*

By H. W. LOY, JR., J. F. HAGGERTY, and O. L. KLINE (Division of Nutrition, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

A Study Panel was appointed by the United States Pharmacopeia in 1949 to consider the possibility of selecting the best features of methods that had been proposed, for the microbiological assay for vitamin $B_{12}$ and incorporating them into a method that would better serve the needs of the Pharmacopeia. After discussion of the problem, laboratory investigation of certain basal medium constituents was investigated. Reported here are the more significant observations made in this laboratory.

The first crystallization of vitamin $B_{12}$ was closely dependent upon the method of assay described by Shorb (6) in which *Lactobacillus lactis* was the test organism; Capps *et al.* (1) demonstrated that *Lactobacillus leichmannii* is also a suitable test organism for this purpose. It appeared that the major difficulty in the standardizing of procedures was that the $B_{12}$ requirement of these organisms varied with change in oxygen tension of the media. The use of the agar plate procedure, although overcoming in some degree this variation, was regarded as unsuitable on other grounds. After reviewing the merits of the procedures used in the

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 1–3, 1951.

first U.S.P. Collaborative Study on $B_{12}$, the following studies were undertaken and results as obtained were reported to the Study Panel.

*Organism.*—Several different strains of organisms have been used in the various microbiological assays for vitamin $B_{12}$. The most commonly used organisms for this assay are: *L. leichmannii*, A.T.C.C. Nos. 4797 and 7830; and *L. lactis*, A.T.C.C. Nos. 8000, 10697, and 10705. In our study of assay methods for vitamin $B_{12}$, the organism *L. leichmannii*, A.T.C.C. No. 7830, appeared to be at least as satisfactory as any of the other test organisms proposed, and is the one used throughout.

*Inoculum Medium.*—Several different media have been described for preparing the stock and inoculum cultures of the test organism. It was found that many of those media that contained skim milk or a skim-milk broth as one of the ingredients were satisfactory with respect to growth of the organism, but difficulty was encountered, in the assay technique, in handling the "curd" that was formed. Thus, it was difficult to separate the test organism from all traces of foreign precipitate and to obtain a uniform suspension of the organism. An inoculum medium that produces consistently good and uniform growth consists of a water solution of yeast extract, peptone, dextrose, potassium phosphate, tomato juice, and polyoxyethylene sorbitan monooleate (Tween 80). The stock culture medium is prepared by the addition of agar to the inoculum medium. In washing the inoculum culture of *L. leichmannii*, it was found that exposure to saline had a deleterious action on the organism. This is in agreement with the observations of Shaw on *L. lactis* (5). To overcome this, the inoculum is prepared from a culture of *L. leichmannii* by repeated washing and final suspension (1:10 dilution) in a solution of sterile basal medium. It was found that the growth of the organism in this medium was excellent, and produced, at the end of a 16-hour incubation period a suspension having a turbidity equivalent to 1.5–2.0 per cent transmittancy at 550 millimicrons. When diluted 1:10, the transmittancy was about 40 to 50 per cent. (See following for method of turbidity measurement.)

*Basal Medium.*—The outstanding characteristic of the writers' first attempts to use the various published methods of assay was the inconsistency in the amount of vitamin $B_{12}$ required to promote maximum growth response. Variations occurred not only between methods, but between assays with the same procedure. It was apparent that the basal medium was nutritionally incomplete and in an attempt to supply unrecognized nutritional factors, a norit-treated tomato juice was found to be more satisfactory than the n-butyl alcohol-extracted asparagus juice used as a supplement by several investigators. Also, norit-treated tomato juice was more effective than the 80 per cent alcohol-precipitated potato extract fraction of Shaw (5) or an 80 per cent alcohol-precipitated tomato juice extract fraction, although these fractions supplied a growth factor

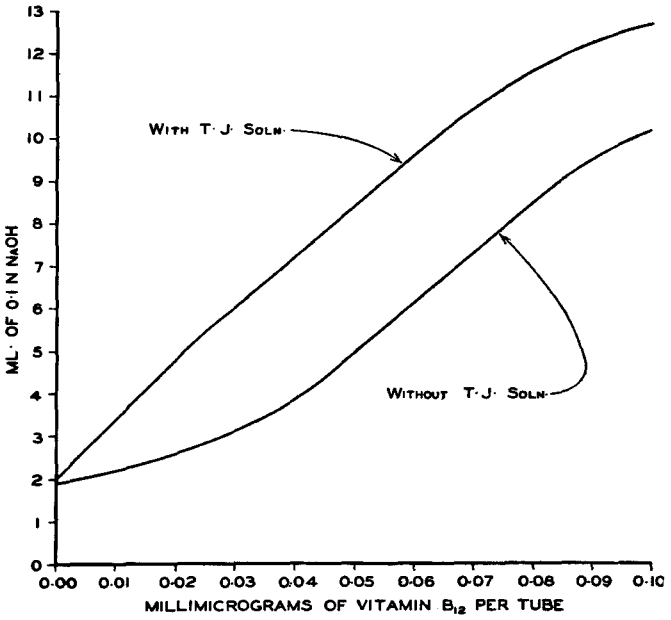


FIG. 1.—Growth response of *L. leichmannii* to vitamin $B_{12}$ with and without the addition of 1.0 ml of a norit-treated tomato juice solution to the basal medium (incubated 72 hours at 34°C.).

that enhanced the plain basal medium. The best and most consistent response was obtained with 1.0 ml of norit-treated tomato juice per tube. The supplementing effect of this tomato-juice solution is demonstrated in Figure 1. (See following for description of the titration method of assay.) Very little difference in response was obtained between different brands of canned tomato juice, or between canned or fresh tomato juice. Upon storage for several months in a refrigerator, norit-treated tomato juice solution retained its growth-promoting activity.

Even with the addition of the tomato juice preparation, reproducibility and uniformity of response were not acceptable. Attention was then focused on hydrolyzed casein as the source of amino acids. It was found that a better response curve was obtained upon addition of casein hydrolysate to a medium in which the amino acids were supplied in the pure form. The addition of nineteen amino acids, singly or combined, did not appreciably supplement the casein hydrolysate. A norit-treated commercial yeast extract[1] was found to supplement a synthetic amino acid medium. While excellent results were sometimes obtained, it was found that inhibition of growth could occur from a slight excess of yeast

[1] Difco yeast extract.

over the optimum amount necessary. Casein hydrolysate gave more consistent results and was used in preference to yeast extract.

A careful study of each individual ingredient of the basal medium was then made. In the method developed, para-aminobenzoic acid was not always required for maximum growth of the organism. On the other hand, folic acid was always essential for maximum growth, though 50–75 per cent of maximum growth was sometimes obtained without it. The addition of 0.1 microgram of folic acid per tube allowed satisfactory growth with no additional response when up to 10 micrograms were added. Amounts in excess of 10 micrograms per tube caused increasing inhibition of growth, particularly in the lower assay levels. These findings are in agreement with those of other workers (2) (7). One microgram of folic acid per tube was selected as a desirable level. The other basal medium ingredients were also tested as to minimum amounts required for optimum growth and maximum amounts allowable without causing inhibition. Each was adjusted to the proper amount with the allowance of an ample safety factor in regard to minimum and maximum.

*Effect of Reducing Agents.**—The variation in the requirement of the

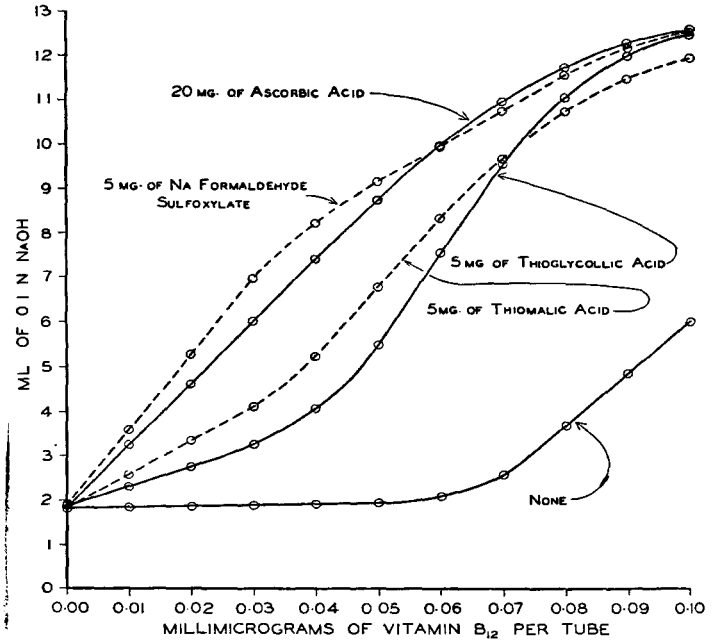


FIG. 2.—Effect of reducing agents (mg per tube) on the growth response of *L. leichmannii* to vitamin $B_{12}$ (incubated 72 hours at 34°C.).

* From a dissertation submitted by J. F. Haggerty in partial fulfillment of the requirements for the degree Master of Science in the Department of Biochemistry, Georgetown University.

organism for vitamin $B_{12}$ was not reduced by the modifications thus far described. Because it had been suggested that the $B_{12}$ requirement was related to the oxygen content of the medium, an attempt was made to use several systems that influence oxidation-reduction potential.

Thioglycolic and thiomalic acids, in use by other workers, were found to reduce the amount of $B_{12}$ necessary to produce a maximum growth response. They were unsatisfactory, however, from the standpoint of stability to changes in *p*H and sterilization time. Other reducing agents, including the succinic-fumaric acid system, ferrous sulfate-cysteine mixture, sodium formaldehyde-sulfoxylate, and ascorbic acid were studied. Of these, sodium formaldehyde-sulfoxylate, thiomalic, thioglycolic, and ascorbic acids were most satisfactory, and their effects upon growth response, compared to that on the plain basal medium, are shown in Figure 2. The addition of five milligrams per tube of sodium formaldehyde-sulfoxylate supported excellent growth, as did the presence of twenty milligrams of ascorbic acid per tube. However, sterilization beyond three minutes resulted in an inhibition of growth, presumed to be due to a breakdown of sodium formaldehyde-sulfoxylate to produce toxic compounds. The amount of ascorbic acid required to produce optimal results was determined after considerable study. Figure 3 shows typical response curves for various levels of added ascorbic acid. Twenty milligrams of ascorbic acid per tube, with its wide margin of safety, was selected as a satisfactory amount for routine use.

It seems probable that the suitability of ascorbic acid for this purpose is dependent upon its maintaining a proper oxidation-reduction potential in the medium throughout the incubation period. Although it is difficult to determine the true oxidation-reduction potential of a solution as complex as the basal medium, approximate EMF values at the beginning and at the end of the incubation period showed little change in the presence of ascorbic acid. It was found possible to maintain a satisfactory potential with several other reducing agents, but it was not possible to control closely the inhibiting effect that frequently occurred upon the addition of amounts necessary to maintain an optimum potential. Ascorbic acid, throughout a wide range in concentration, did maintain a uniform potential without any inhibiting effect.

*p*H *of the Medium.*—It was observed that small changes in the *p*H of the basal medium sometimes resulted in considerable variation in growth response. A detailed study was then made of the effect of varying the *p*H from 5.0 to 7.2. At *p*H above 6.9 growth was suppressed, and best growth response was obtained consistently at *p*H 6.0. In the titration procedure the titratable activity of 10 ml of the final medium is approximately 1.7 ml and in our experience titration values for the inoculated blank tubes are in the range of 1.7 to 2.5 ml.

When the reducing agent and other ingredients of the basal medium

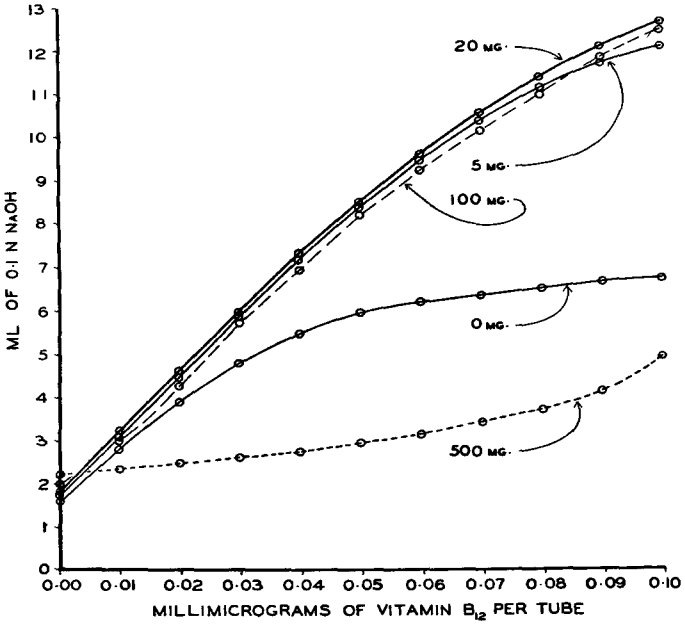


FIG. 3.—Effect on growth response of *L. leichmannii* to vitamin $B_{12}$ when varying amounts of ascorbic acid (mg per tube) are added to the basal medium (incubated 72 hours at 34°C.).

were held to correct amounts, a consistent maximum growth response between assays resulted. Adventitious inhibition or stimulation of growth in the assay tubes was almost completely eliminated by a refined technique of washing and care of glassware (3). In addition it was found that heating these specially cleaned tubes in an oven at 180°–200°C. for two hours eliminated still further the possibility of contamination.

The vitamin $B_{12}$ present in each tube can be estimated either by turbidity or by titration measurement.

*Turbidity Measurement.*—For turbidity measurements the following procedure has been adopted. After a 16-hour incubation period the contents of the tubes are transferred to previously matched (within $\pm 0.5\%$) Pyrex test tubes (No. 9800, 18×150 mm). The per cent transmittancy is then measured at a wave length of 550 millimicrons in a Beckman Spectrophotometer (Model B) fitted with a test-tube adapter. Difficulty is encountered in correcting for the sample "blank" with some products of low potency. This is due to the presence of extraneous color or foreign turbidity that may vary with acidity in the assay tubes.

*Titration.*—For titration, the contents of each tube are titrated directly

to an end point of 6.8 measured electrometrically with a Beckman *p*H meter (Model H-2), equipped with special glass and calomel electrodes approximately 175 mm in length and 5 mm in diameter. The electrodes, plus a thin tube (through which purified air is passed to agitate contents) fit directly into the tube, permitting rapid and accurate measurement of *p*H.

## DISCUSSION

Both methods are currently employed in our laboratory and to demonstrate the growth correlation in response obtained by these two methods, reference is made to the curves of Figure 4.

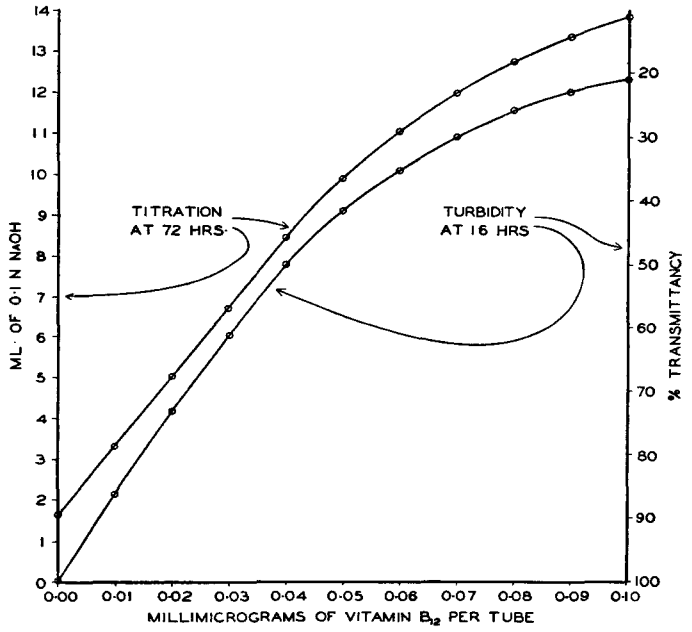


FIG. 4.—Growth response of *L. leichmannii* to vitamin $B_{12}$ (incubated at 34°C.).

Under the conditions described here, the requirement of the test organism for vitamin $B_{12}$ has been found to be remarkably uniform from one assay to another. The need for careful control of the conditions of the assay, and for meticulous technique in preventing contamination should be emphasized. The use of 0.01 to 0.1 millimicrogram of the vitamin in the assay tubes makes possible a great dilution of most sample solutions, thereby diluting out the inhibiting or stimulating effect of most interfering substances.

## CONCLUSIONS

A procedure that included the modifications discussed here was submitted to the U.S.P. Study Panel on Vitamin $B_{12}$. With some additional minor modifications the procedure was the subject of collaborative study under the auspices of the U.S.P. Anti-Pernicious Anemia Preparations Board and results of that study, as yet unpublished, served as a basis for adoption of the method that now appears in the Third Supplement of U.S.P. XIV. The same procedure, with minor modifications, has also been used in the A.O.A.C. 1951 Collaborative Study for the Assay of Vitamin $B_{12}$ activity of feeds.

More recent experience, in both this and other laboratories, has indicated that optimum growth may be attained using the basal medium without the tomato juice solution. This occurs when the test organism, maintained on tomato juice agar medium, is in a highly activated state. Behavior is not consistent, however, and we are unable to predict whether or not stimulation may result from the addition of tomato juice to the medium.

The finding that vitamin $B_{12}$ and $B_{12b}$[2] are converted during the sterilization process to a form more available to the organism (4) has made possible the use of crystalline $B_{12}$ as a Reference Standard. It will be of interest to record that 25 per cent alcohol solutions of crystalline vitamin $B_{12}$ of concentrations varying from one millimicrogram to twenty micrograms per ml, stored at 10°, have retained their full activity over a period of more than eighteen months.

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[2] We are grateful to Dr. D. V. Frost, Abbott Laboratories, for a generous supply of vitamin $B_{12b}$ solution, and to Dr. A. B. Scott, Merck and Co., for crystalline vitamin $B_{12}$ used in these studies.

# STABILITY OF VITAMIN $B_{12}$ AND $B_{12b}$*

By H. W. Loy, Jr., J. F. Haggerty, and O. L. Kline (Division of Nutrition, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Vitamin $B_{12}$ and $B_{12a}$ have been identified chemically as cyano- and hydroxo-cobalamin by Folkers and his associates (1). It has been shown further by physical characteristics that vitamin $B_{12a}$ and the $B_{12b}$ isolated by the Lederle group (2) are identical (3). There is evidence from several directions that the naturally occurring form of the vitamin is closely similar to, or is predominantly the hydroxo- form. Lang and Chow found vitamin $B_{12}$ to be unstable when heated in the presence of reducing agents (4). Frost and co-workers (5) demonstrated a differential stability between $B_{12}$ and $B_{12b}$ in the presence of ascorbic acid and proposed its use as a means of differentiating $B_{12b}$. In our study of the A.O.A.C. collaborative samples described by Dr. Krieger in his 1951 report,† we observed large differences in vitamin $B_{12}$ activity of each sample when the various extraction procedures were applied. It was our experience, and, judging from Dr. Krieger's report, that of other collaborators as well, that the inclusion of bisulfite in the extracting medium yielded an extract of higher vitamin $B_{12}$ activity in every case. Frost and associates (6) observed an increased stability of $B_{12b}$ in the presence of bisulfite, and employed the term sulfito-cobalamin to describe the complex.

Kaczka *et al.* (7) pointed out recently that cobalamin combines with ions other than cyano- or hydroxo- and demonstrated the formation of chloro, sulfato, nitro, and other forms of cobalamin. It is possible that some of these forms possess greater stability to heat than does the naturally occurring hydroxo-cobalamin.

Because of the apparent loss of naturally occurring vitamin $B_{12}$ activity during the extraction process it seemed desirable to establish the effect of variation of *p*H and of time of heating upon $B_{12}$ and $B_{12b}$ with and without the use of bisulfite.

## EXPERIMENTAL AND RESULTS

In view of the report (5) of the destructive effect of ascorbic acid upon vitamin $B_{12b}$, we were concerned with its stability in the basal medium of the microbiological assay which contains 20 mg of ascorbic acid per tube. For this study we had available a solution of vitamin $B_{12b}$ obtained from Dr. H. H. Fricke of Abbott Laboratories, who prepared it from crystalline $B_{12b}$ of at least 92% purity. The vitamin $B_{12}$ we used was a crystalline preparation of high purity, obtained from Dr. A. B. Scott of Merck and Co. A series of tubes containing 10 millimicrograms of $B_{12}$

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 1–3, 1951.

† Associate Referee report, to be published in *This Journal* at a later date.

or $B_{12b}$ in 10 ml of medium, made up asceptically with different amounts of ascorbic acid or thioglycolate, were allowed to stand 0, 24, and 48 hours at room temperature in the dark. The vitamin content was then measured by the U.S.P. microbiological method (9). The amount of vitamin in the stored tubes was 10 to 100 times the amount normally added to the standard series of tubes in the assay. For comparison, mixtures of 100 mg of ascorbic acid and 100 millimicrograms of $B_{12}$ or $B_{12b}$ in dilute acid solution were allowed to stand under the same conditions. Results are presented in Table 1. Under conditions described for the basal medium,

TABLE 1.—*Stability of vitamins $B_{12}$ and $B_{12b}$ to reducing agents in the basal medium and to ascorbic acid solution*

| TREATMENT | VITAMIN | TIME (HRS.) | | |
|---|---|---|---|---|
| | | 0 | 24 | 48 |
| | | *per cent remaining* | | |
| Basal Medium (no reducing agent) | $B_{12}$ | 100 | 100 | 100 |
| | $B_{12b}$ | 100 | 100 | 12 |
| Basal Medium+1.5 mg Ascorbic Acid | $B_{12}$ | 100 | 100 | 100 |
| | $B_{12b}$ | 100 | 100 | 74 |
| Basal Medium+20 mg Ascorbic Acid | $B_{12}$ | 100 | 100 | 100 |
| | $B_{12b}$ | 100 | 87 | 75 |
| Basal Medium+7.5 mg Thioglycollate | $B_{12}$ | 100 | 100 | 100 |
| | $B_{12b}$ | 100 | 76 | 76 |
| Ascorbic Acid Soln—100 mg | $B_{12}$ | 100 | 89 | 87 |
| | $B_{12b}$ | 100 | 10 | 0 |

$B_{12}$ remained unchanged throughout the 48-hour period. Some loss was observed in the $B_{12}$ and ascorbic acid solution after 24 hours. In contrast, $B_{12b}$ was destroyed to a marked degree after 48 hours in the basal medium without reducing agent. A measurable loss occurred in the medium containing reducing agent after 48 hours standing. That such loss does not occur during the incubation period of an assay is demonstrated by the fact that identical values for $B_{12b}$ potency, in terms of the reference standard $B_{12}$, have been obtained after 16 hours and after 72 hours of incubation. Although an explanation of this difference is difficult, it may be related to conversion of both $B_{12}$ and $B_{12b}$ to another and perhaps more stable form during the sterilization step (8). Vitamin $B_{12b}$ was found to be quite unstable in the presence of ascorbic acid in pure solution.

The effect of heat at *p*H 4.5 on stability of $B_{12}$ and $B_{12b}$ with and without bisulfite is shown in Table 2. The solutions heated at 121–123°C. for the indicated periods contained 3.3 millimicrograms of $B_{12}$ or $B_{12b}$ per

ml in a citrate-phosphate buffer. The *p*H of these solutions was found to be the same before and after the heating period. For bisulfite addition, a concentration 0.1 per cent of sodium metabisulfite was used. It should be noted that although both $B_{12}$ and $B_{12b}$ showed a loss of potency, particularly with the longer heat treatments, they were completely stable in the presence of bisulfite.

Following these observations we determined stability of the vitamins to heat over a wide range of *p*H, with and without addition of bisulfite. Solutions having a range of *p*H from 1.0 to 9.0 were prepared as follows: for *p*H 1.0, 0.11 *N* HCl; for *p*H 2.0, 0.1 *M* citric acid+trace of HCl; for *p*H 2.5 to 8.0, varying proportions of $Na_2HPO_4$ and citric acid; and

TABLE 2.—*Effect of autoclaving in buffered solution at* pH *4.5 upon vitamin* $B_{12}$ *and* $B_{12b}$ *activity*

| VITAMIN | AUTOCLAVING TIME IN MINUTES | | | |
|---|---|---|---|---|
| | 20 | 30 | 60 | 120 |
| | *per cent remaining* | | | |
| $B_{12}$ | 98–102 | 97–101 | 89–92 | 60–64 |
| $B_{12b}$ | 73–82 | 59–61 | 30–32 | 14–15 |
| $B_{12}$+Bisulfite | 98–100 | 98–100 | 98–101 | 99–100 |
| $B_{12b}$+Bisulfite | 99–103 | 99–102 | 100–101 | 98–101 |

for *p*H 9.0, 0.1 *M* $Na_2HPO_4$+trace of NaOH. Results in Table 3 indicate that $B_{12}$ heated 20 minutes at 121°–123°C. is completely unchanged from *p*H 2.5 to 7.0. There is marked loss above and below these points. The greater lability of $B_{12b}$ is quite apparent; in fact under these conditions of heating it is stable only in the narrow range of 2.5 to 3.5. In marked contrast, in the presence of bisulfite the two vitamins have an identical and complete stability from *p*H 2.5 through 9.0. Results on unbuffered solutions, included in Table 3, demonstrate that the added buffer was without effect with respect to the change in potency.

To demonstrate the degree of protection conferred by the bisulfite addition, solutions of the two vitamins with bisulfite, covering a *p*H range of 2.0 to 9.0, were heated 2 hours at 121°–123°C. Results presented in Table 4 show again the similarity of behavior of the two vitamins. In the presence of bisulfite they completely retained their potency from *p*H 3.5 to 9.0, but below *p*H 3.5 there was a progressive loss.

A comparison of these results with results of a study of the vitamin in natural materials is of interest. In Table 5 are presented assay data for solutions of skim milk and liver injection, adjusted to *p*H 1.0, 3.0, 4.5, and 6.7, heated 20 minutes and 2 hours, and in the latter case with and without bisulfite. The vitamin $B_{12}$ activity of the skim milk used was 3.6 micrograms per liter, and of the liver injection 10.5 micrograms

TABLE 3.—*Stability of vitamins $B_{12}$ and $B_{12b}$ to 20-minute autoclaving in buffered solutions of varying* pH

| VITAMIN | pH (AT 25°C.) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.0 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.4* | 4.5 | 6.0 | 7.0 | 7.0† | 8.0 | 9.0 |
| | *per cent remaining ranges* | | | | | | | | | | | | |
| $B_{12}$ | 30–31 | 59–100 | 98–100 | 99–102 | 100–103 | 96–100 | | 98–102 | 100–102 | 98–100 | 100–101 | 35–37 | 20–22 |
| $B_{12b}$ | 26–28 | 57–99 | 98–103 | 98–103 | 98–100 | 83–85 | | 73–82 | 55–66 | 48–57 | 47–51 | 28–31 | 18–22 |
| $B_{12}$+ Bisulfite | 36–37 | 62–101 | 99–100 | 99–102 | 100–102 | 99–101 | 96–100 | 98–100 | 99–101 | 99–101 | | 98–100 | 100–102 |
| $B_{12b}$+ Bisulfite | 32–37 | 60–100 | 98–103 | 99–101 | 100–103 | 99–101 | 99–101 | 99–103 | 99–102 | 98–103 | | 99–100 | 99–102 |

* No buffer ($H_2O$ only + bisulfite + vitamin).
† No buffer ($H_2O$ only + vitamin).

TABLE 4.—*Effect of 120-minute autoclaving in buffered solutions containing 0.1% sodium metabisulfite on vitamin* $B_{12}$ *and* $B_{12b}$ *activity*

| VITAMIN | pH (AT 25°C.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 6.0 | 7.0 | 8.0 | 9.0 |
| | | | | | *per cent remaining* | | | | | |
| $B_{12}$+ Bisulfite | 8–21 | 47–53 | 93–102 | 99–100 | 99–102 | 99–100 | 97–100 | 98–100 | 97–100 | 98–103 |
| $B_{12b}$+ Bisulfite | 8–20 | 45–53 | 91–100 | 99–101 | 99–100 | 98–101 | 100–104 | 100–101 | 96–100 | 100–103 |

TABLE 5.—*Stability of natural vitamin $B_{12}$ activity in skim milk and liver injection to 20- and 120-minute autoclaving*

| PRODUCT | AUTOCLAVING TIME IN MINUTES | *p*H (AT 25°C.) | | | |
|---|---|---|---|---|---|
| | | 1.0 | 3.0 | 4.5 | 6.. |
| | | *per cent remaining* | | | |
| Skim Milk | 20 | 14–17 | 46–59 | 38–58 | 33–47 |
| Liver Injection | 20 | 0–2 | 53–82 | 47–68 | 12–20 |
| Skim Milk | 120 | 0–2 | 10–15 | 6–10 | 0–5 |
| Liver Injection | 120 | 0–3 | 20–25 | 18–24 | 0–5 |
| Skim Milk + Bisulfite | 120 | 0–5 | 98–101 | 100–102 | 11–15 |
| Liver Injection + Bisulfite | 120 | 0–4 | 80–90 | 97–100 | 99–102 |

per ml. With 20-minute heating there is marked loss of vitamin potency in both materials, and the degree of loss with *p*H change is greater than that obtained for the $B_{12b}$ solutions. Increased destruction occurred with the longer heat treatment. The protective effect of bisulfite is again apparent; protection at *p*H 3.0 was nearly complete, as it was for the $B_{12b}$ solution (Table 4). The difference in potency between the two materials heated at *p*H 6.7 is difficult to explain. The milk suspension showed marked browning, and had much more solid material in suspension than the solution containing the liver injection.

The extractions of such materials as milk or liver products involving short heating appear to be safe if it is carried out in the presence of bisulfite at *p*H 4.5. The concentration of sodium metabisulfite used in these studies was 0.1 per cent. Ordinarily, because of the small amounts of vitamin $B_{12}$ needed in the assay tubes, the sample solution containing bisulfite is diluted many fold and inhibition of growth of the organism is not a problem. However, a 0.1 per cent solution of bisulfite added directly to the assay tubes is inhibiting, and it is suggested that the final concentration should be not greater than 0.001 per cent bisulfite.

## SUMMARY

(1) Although under some conditions vitamins $B_{12}$ and $B_{12b}$ show a loss of potency in the presence of reducing agents, they are stable in the basal medium containing ascorbic acid as used in the U.S.P. microbiological assay.

(2) Vitamin $B_{12b}$ is much less stable to heat than $B_{12}$ over a wide range of *p*H.

(3) In the presence of bisulfite and under conditions of drastic heating, vitamins $B_{12}$ and $B_{12b}$ show identical and complete stability from *p*H 3.5 to 9.0.

(4) Stability to heat of vitamin $B_{12}$ activity of skim milk and liver injection is greater in the presence of bisulfite, and protection was found to be complete at *p*H 4.5.

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. A. B. Scott, Merck and Company for generous amounts of crystalline vitamin $B_{12}$, and to Dr. D. V. Frost and H. H. Fricke, Abbott Laboratories, for a supply of vitamin $B_{12b}$.

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## BOOK REVIEWS

**Elements of Plant Protection.** By LOUIS L. PYENSON, Entomologist and Plant Pathologist, Long Island Agricultural and Technical Institute. 8vo., cloth, 536 pp., 226 illus., with line drawings by Emily B. Steffens. N. Y., John Wiley & Sons, Inc., London, Chapman & Hall, Ltd., 1951. $4.96.

This volume has been prepared as a text and source book for students in vocational agriculture classes in high schools, in technical institutes, and in the early years of agricultural colleges. In it an attempt has been made to bring together the most helpful information that pertains to the status and control of the most important and widely distributed plant enemies, notably: insects, disease-producing organisms, rodents, birds and weeds. The material has been organized to make it adaptable for introductory one-semester courses in economic entomology, plant pathology, or weed control; or for one continuous course covering all three of these subjects. The subject matter also has been arranged so that certain parts may be omitted, if the nature and length of the course so dictate. Questions have been arranged at the end of each chapter for convenience in class use and to stimulate discussion. The last three chapters on the use of application equipment have been made equally applicable to all common phases of plant protection. A selected bibliography of useful reference works from which much of the material was gleaned, as well as a glossary of 8 pages have been included. The wide scope of the subject matter of the book forbids detailed discussion or inclusion of very much specific information on the many subdivisions of the subject. Such as has been given has been mainly for purpose of illustrating types. The main objective has been rather to lay groundwork for subsequent field or laboratory work on identification and control of specific pests within range of the student's own activities. Although intended mainly for students, an attempt has been made to render the book of service as a basic reference work to all those in agricultural pursuits.

J. S. WADE

**Insect Control by Chemicals.** By W. A. BROWN, Ph.D., Professor and Head, Department of Zoology, University of Western Ontario. Small 8vo., cloth, 817 pp., illus., tables, index. N. Y., John Wiley & Sons, 1951, $12.50.

During the first half of the twentieth century the rapid development of our knowledge of chemical insecticides has resulted in large part from extensive-scale farming practices, and has stimulated the recent appearance of this as well as several other exceedingly worthwhile books on the subject. In the early years of this period the available materials included the arsenicals, lime-sulphur, petroleum oils, and nicotine. During the interval between World Wars I and II, the fluorine compounds were added to the inorganics, pyrethrum and rotenone were added to the botanicals, and synthetic organic materials such as the dinitro compounds and thiocyanates made their appearance. The coming of World War II witnessed the rise of the chlorinated hydrocarbon insecticides, with DDT contributed by Switzerland and BHC by the United Kingdom and France; their subsequent development in the United States was followed by the appearance of toxaphene, chlordane, aldrin, and dieldrin. Meanwhile, German work during the war produced a powerful group of insecticides, viz., the organic phosphates, among which TEPP and parathion were produced commercially in the United States, and the systemic insecticides were developed in the United Kingdom. As the century reaches the halfway mark, emphasis is returning to the eminently suitable pyrethrins, whose toxicity is being extended by admixture of the piperonyl compounds, a synthetic analogue of which has appeared in the form of allethrin.

An excellent idea of the general scope of this book may be gained by consideration of its various main subdivisions. These comprise such subjects as (1) Insecticides of the Mid-Twentieth Century and Their Properties. (2) The Structure of Organic Chemicals and Their Toxicity to Insects. (3) Susceptibility of Insects to the Entry of Poisons. (4) The Pharmacology of Poisons for Insects. (5) Equipment Developed for the Application of Insecticides. (6) The Application of Insecticides from Aircraft. (7) Toxicity and Hazards to Man and Domestic Animals. (8) Toxicity of Insecticides to Plant Growth. (9) Chemical Control of Insects Feeding on Plants. (10) Chemical Control of Insects Affecting Man and Animals and (11) Insecticides and the Balance of Animal Populations.

The insecticides in common present-day use are classified and their chemical and physical properties are indicated. In addition, the full range of organic compounds is covered in the light of insecticide testing, and the most active groups are given special consideration. The mode of action of insectides is discussed from the physiological and toxicological viewpoints, and there is full treatment of the hazards to be avoided in formulation, mixing, and use of toxic compounds and of their effects on plants, on domestic animals, and on man. Also, consideration is given to lethal dose, symptoms caused by accidental poisoning, and antidotes and precautionary measures. A section likewise is devoted to effects of various insecticides on wild animals, changes in the balance of nature and the appearance of resistant insect strains.

It is noteworthy that in this book particular attention is given to the most modern application equipment. Copiously illustrated, the discussion includes such items as various types of nozzles, compressed-air sprayers, hydraulic sprayers, bucket pumps, knapsack, barrel pump, orchard power and boom sprayers, air atomizers, paint and electric sprayers and blowers, aerosol generators, hot gases, steam, rotating discs, explosive bombs, liquefied gas, smokes, fumigators, dusters, bait spreaders and the like. Down-to-date information of this kind is of enormous practical value to entomologists, insecticide chemists, pest control operators, aerial

crop sprayers, comparative physiologists, manufacturers and salesmen of agricultural chemicals and many others. The book also covers such matters as the physics of spraying and dusting in relation to latest use of aircraft in insect control and the history, application, advantages and disadvantages. The various installations for spraying, dusting and baiting likewise are illustrated and discussed, and comparative merits of helicopters and fixed-wire aircraft in insecticide work are pointed out.

The book is based upon a background of more than twenty years of practical experience in insect physiology and toxicology in Europe and America with much of it in active field work and research assignments. In addition to several years with the Canadian Chemical Warfare Directorate, and research on Royal Society of Canada Fellowship under Dr. V. B. Wigglesworth at the University of London, the author has served as Head of the Entomological Branch, Canadian Forest Insect Survey; Head of the Entomology Section, Suffield Experiment Station, Defense Research Board; and Entomologist on Northern Canadian work with its Department of Agriculture.

Dr. Brown may well be congratulated upon the successful completion of this difficult and important work. It is certain to have a wide usefulness.

J. S. WADE

**The Vitamin B Complex.** By F. A. ROBINSON. John Wiley & Sons, Inc., 440 Fourth Ave., New York, N. Y. 1951. 688 pages. Price $9.00.

The author has participated actively in this field since 1935, and his extensive survey of all the factors that have been included in the group of B vitamins is proof of his thorough familiarity with the subject.

In presenting and correlating the published information the eleven well-characterized B vitamins are treated separately, each under 15 to 20 headings. The different sections deal with such subjects as isolation, chemical constitution, synthesis, properties and stability, methods of determination, occurrence, effects of deficiency and animal and human requirements, metabolism, relation to microorganisms, plants and insects, and analogues. Each section is followed by a list of references, which, although not complete in all instances, will be helpful to those actively engaged in the field.

A more or less complete discussion of methods of synthesis (with patent references) for most of the factors is presented. A detailed discussion of metabolic relationships with enzyme systems, for those vitamins known to act as coenzymes, will be of interest to investigators concerned with the function of the vitamins. The inclusion of a summary of knowledge concerning analogues and anti-vitamins is an important feature. Such analogues are coming into use in medicine, and clinicians as well as biochemists will find this summary helpful.

The bibliographies include references to papers published as late as 1949, which may be regarded as up-to-date for a volume as extensive as this.

Those unfamiliar with this field and unaware of the great amount of experimental work that has been published will find this review impressive and presented in a style to hold their interest. In the author's words he has "satisfied his urge to systematize the heterogeneous, rather untidy, array of data, so that others not so intimately acquainted with the field may have an over-all picture of what the vitamin B complex is and why it is of such significance in human and animal nutrition and in the economy of microorganisms."

Those who are specialists in the subjects covered may criticize the inclusion or omission of some references, or may differ with the author regarding the emphasis

placed on some. However, the general development of information with respect to each of the B vitamins appears to be accurate.

O. L. KLINE

**Industrial Water Pollution—Survey of Legislation and Regulations.** By MARVIN D. WEISS of R. S. Aries and Associates. Chemonomics, Inc., 400 Madison Ave., NewYork 7, N. Y. 1951. IV+142 pages, lithoprint, paper bound. Price, $5.00.

Because of the increasing health risks and the varying industrial use problems, public health and other regulatory agencies have given additional attention during the past decade to preventing and correcting the polluted condition of our waterways. The wasteful use of our underground water reserves is recognized. Controls by the Federal and State Governments have advanced so rapidly that industry finds it difficult to keep informed of existing requirements.

From replies, received from all 48 States (except Colorado and Wyoming), the District of Columbia, Hawaii, Puerto Rico, Alaska and the Virgin Islands, a comprehensive survey of the current legislation and regulations has been organized, with code references. For orientive purposes, the Federal Standard Legislation is discussed in some detail. A record of the activities of interstate agencies for the control of coastal and fresh water pollution is included.

In many instances waters have been classified according to their use, and quality standards have been established for each class. The controls vary in each State according to industrial activities and, in addition to basic requirements for potable supplies, may include those for (1) recreational areas (swimming and bathing) in both salt and fresh waters, (2) fish and aquatic life in both salt and fresh waters, (3) wild life preserves, (4) irrigation (truck crop gardening, fruit trees, or for general use), (5) industrial for food preparation before processing. (6) industrial for cooling operations (exclusive of direct contact with food), (7) wastes from food processing plants, and (8) refuse from mining, oil and gas well drilling, paper manufacturing, saw mill operations, etc.

Regulations may be for the control of (1) the quantity and type of discharge either before or immediately after leaving the industrial or other source (intended to avoid unfair commercial advantage among pollutors), and/or (2) the residual contamination at different locations in the effluent areas after various agents have exercised their dissipating action. The goal, both nationally and State-wide, is a plan organized in the interests, first, of public health safety and, second, of equitable industrial use to prevent, abate and control all forms of pollution and contamination of underground, surface, and coastal waters, to conserve water supplies, and to reduce erosion hazards in crop and forest areas.

Even in abstract form, the subject matter is broader than the title of the book indicates. The Appendix includes:

I. Tentative Standards for Water Classifications—New England Interstate Water Pollution Control Commission.
II. Water Classification Standards—Delaware River Basin.
III. Criteria for the Classification of Streams—Potomac River Basin.
IV. Standards for Treatment of Sewage—Interstate Sanitation District.
V. Membership of Water Pollution Control Commissions.
VI. Identification List of State Water Pollution Agencies.

Public Health, and Food, Drug and Cosmetic regulatory workers should know that these water pollution control agencies exist and that in many instances limiting standards for water pollution have been established.

A. H. ROBERTSON

**Vitamin Methods, Volume II. (1951).** By PAUL GYORGY. Editor Academic Press, Inc. 125 East 23rd Street, New York 10, N. Y. xi+740 pages. Price $14.50.

This volume is concerned primarily with animal assay methods for the vitamins. Animal assays have been indispensable in developing our present knowledge of the vitamins, and although, for control purposes they have been largely replaced by the physical, chemical and microbiological techniques described in Volume I, they will unquestionably retain their importance as the final criterion of specificity in vitamin research—and they continue to be the only reliable means for estimating certain dietary essentials.

The subject is introduced by N. B. Guerrant with a chapter (40 pp.) on the general aspects of small animal experimentation. C. I. Bliss and P. Gyorgy present the animal vitamin assays in a section that is of monograph proportions (234 pp.). A chapter (165 pp.) on statistical methods in vitamin research, by C. I. Bliss, is closely correlated with the chapter on assay methods. Interspersed are chapters on the laboratory diagnosis of human vitamin deficiencies by J. H. Jones (118 pp.) and a short chapter (40 pp.) on the clinical signs of malnutrition by N. Jolliffe. The last 100 pages constitute, in three sections, a supplement to Volume I and cover the developments that were omitted in Volume I in the physical, chemical and microbiological methods of assay up to 1949–50.

The authors of the various chapters are well known to all engaged in the field of vitamin research. Experienced workers will enjoy Dr. Guerrant's presentation and for newcomers it should be regarded as essential reading. Dr. Bliss is widely known for his interest in the application of modern statistical theory to biological assay. In the chapter on animal vitamin assays the statistical approach is emphasized.

Following a review of general principles, treatment of each of 13 vitamins includes history and use of standards, basal rations, preparation of animals and experimental design, the test period and types of response used, and interpretation of results. Where more than one type of response or assay method has been used, each is described in detail. The authors make clear throughout the requirements of the U.S.P. and A.O.A.C. procedures.

The chapter on statistical methods has been prepared—"for workers not trained in modern biometrics . . . so that even the inexperienced may make use of modern statistical tools." It undoubtedly will be very useful as a manual.

Under the title of "Laboratory Diagnosis of Human Vitamin Deficiencies," are discussed the methods of vitamin determination in blood and urine. A number of procedures are given in detail. Metabolic products such as lactic and pyruvic acids, serum phosphatase, Ca and P, and prothrombin, are treated similarly. Methods of conducting urinary excretion tests, with special reference to the evaluation of availability to the human of vitamins from various sources, are discussed.

A desirable feature of the book is the extensive list of references at the end of each chapter. Each reference is numbered according to the order of its appearance. A complete author index makes location of the reference to any particular worker very convenient.

Although vitamin assay methods have been the subject of other books and reviews, nowhere have so many methods of interest to the worker in this field been brought together and treated so thoroughly. Dr. Gyorgy and his collaborators have done an excellent job, and this reviewer is certain the book will be a welcome addition to the reference shelf of every worker in the field.

LEO FRIEDMAN