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CONTRIBUTED PAPERS

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PROCEEDINGS OF THE SIXTY-SEVENTH ANNUAL MEETING OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1953

The sixty-seventh annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., October 12, 13, and 14, 1953.

The meeting was called to order by the President, Harry J. Fisher, on the morning of October 12, at 10:00 A.M.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER 1954

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SUBJECTS, REFEREES, AND ASSOCIATE REFEREES†

SUBCOMMITTEE A:

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

CAUSTIC POISONS:

Referee: Harold F. O'Keefe, Food and Drug Administration, Washington 25, D. C.

DISINFECTANTS:

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

FUNGICIDES AND SUBCULTURE MEDIA:

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

MEDIA FOR DISINFECTANT TESTING:

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

SPORICIDAL TESTS:

J. L. Friedl, Production and Marketing Adm., Insecticide Division, Beltsville, Md.

FEEDING STUFFS:

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

CRUDE FAT OR ETHER EXTRACT:

H. H. Hoffman, Department of Agriculture, Tallahassee, Fla.

CRUDE PROTEIN IN FEEDING STUFFS:

E. A. Epps, Jr., La. Department of Agriculture and Immigration, P. O. Box 89, Baton Rouge, La.

DRUGS IN FEEDS

R. T. Merwin, Agricultural Experiment Station, New Haven, Conn.

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GOSSYPOL:

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MICROSCOPIC EXAMINATION:

J. A. Shrader, Agricultural Experiment Sta., Lexington 29, Ky.

MOLASSES:

George T. Peckham, Clinton Foods, Clinton, Iowa

MINERAL CONSTITUENTS OF MIXED FEEDS:

J. C. Edwards, Department of Agriculture, Tallahassee, Fla.

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

SAMPLING:

Bruce Poundstone, Dept. of Feed and Fertilizer, Agricultural Experiment Station, Lexington 29, Ky.

FERTILIZERS:

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

ACID- AND BASE-FORMING QUALITY:

E. W. Constable, State Department of Agriculture, Raleigh, N. C.

AMMONIACAL SOLUTIONS AND LIQUID FERTILIZERS:

J. F. Fudge, Agricultural Experiment Station, College Station, Texas.

BORON:

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

COPPER AND ZINC:

H. J. Webb, A. and M. College of South Carolina, Clemson, S. C.

FREE WATER:

W. L. Hill, Plant Industry Station, Beltsville, Md.

INERT MATERIALS:

K. G. Clark, Division of Fertilizer and Agricultural Lime, Beltsville, Md.

MAGNESIUM AND MANGANESE:

John B. Smith, Agricultural Experiment Station, Kingston, R. I.

NITROGEN:

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

PHOSPHORIC ACID:

K. D. Jacob, Plant Industry Station, Beltsville, Md.

POTASH:

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

SAMPLING AND PREPARATION OF SAMPLE:

Stacey B. Randle, N. J. Agr. Expt. Station, New Brunswick, N. J.

SULFUR:

Gordon Hart, Department of Agriculture, Tallahassee, Fla.

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

AMINO ACIDS:

Francis H. Bird, Westbrook Laboratory, R.F.D. #3, Rockville, Conn.

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CAROTENE:

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

NICOTINIC ACID:

J. P. Sweeney, Food and Drug Administration, Washington 25, D. C.

PANTOTHENIC ACID (MICROBIOLOGICAL METHOD):

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

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

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

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ALDRIN:
S. J. Few, Miss. State Chem. Laboratory, State College, Miss.
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PHENOLIC DISINFECTANTS:
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PHYSICAL PROPERTIES OF ECONOMIC POISONS:
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PIPERONYL BUTOXIDE:
B. L. Samuel, Div. Chemistry, Dept. of Agriculture, Richmond 19, Va.
PYRETHRINS:
D. Kelsey, Production and Marketing Adm., Insecticide Div., Beltsville, Md.
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RODENTICIDES:
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ROTENONE:
R. Payfer, Plant Products Div., Dept. of Agriculture, Ottawa, Canada
SYSTEMIC INSECTICIDES:
Paul A. Giang

VOLATILITY OF ESTER FORMS OF HORMONE TYPE HERBICIDES:
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PLANTS:
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BORON:
E. Truog, Dept. Soils, University of Wisconsin, College of Agriculture, Madison 6, Wis.
CAROTENE:
E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.
COPPER AND COBALT:
Kenneth C. Beeson, U. S. Plant, Soil, and Nutrtional Lab., Ithaca, N. Y.
POTASSIUM:
W. T. Mathis, Connecticut Agricultural Experiment Station, New Haven 4, Conn.
SAMPLING:
E. J. Miller
SODIUM:
Eunice J. Heinen, Mich. Agricultural Experiment Sta., East Lansing, Mich.
STARCH:
Carroll L. Hoffpauir, Southern Regional Research Lab., New Orleans 19, La.
SUGAR:
Kenneth T. Williams, Western Regional Research Lab., Albany 6, Calif.
ZINC:
E. J. Benne

SOIL CONDITIONERS:
Referee: L. T. Alexander, Soil Conservation Service, Plant Industry Station Beltsville, Md.
PERFORMANCE:
S. J. Toth, Rutgers University, New Brunswick, N. J.

SOILS AND LIMING MATERIALS:
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BORON:
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EXCHANGEABLE CALCIUM AND MAGNESIUM:
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EXCHANGEABLE HYDROGEN:
W. M. Shaw
EXCHANGEABLE POTASSIUM:
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HYDROGEN-ION CONCENTRATION OF SOILS:
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PHOSPHORUS:
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ZINC AND COPPER:
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SUBCOMMITTEE B:
HARRY J. FISHER (1954) (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Chairman;* G. R. CLARK (1956); and JONAS CAROL (1958).

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

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

VEGETABLE DRUGS AND THEIR DERIVATIVES:
Referee: P. S. Jorgensen, Food and Drug Administration, San Francisco 2, Calif.
AMINOPHYLLIN AND PHENOBARBITAL:
Rupert Hyatt, Food and Drug Administration, Cincinnati 2, Ohio
AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL:

QUININE AND STRYCHNINE:
D. J. Miller, Food and Drug Administration, Buffalo 3, N. Y.

SYNTHETIC DRUGS:
Referee: F. C. Sinton, Food and Administration, New York 14, N. Y.
ACETOPHENETIDIN AND CAFFEINE (Chromatographic Separation):
Gordon Smith, Food and Drug Administration, New York 14, N. Y.
AMOBARBITAL SODIUM AND SECOBARBITAL SODIUM (TUINAL ®):
George E. Keppel, Food and Drug Administration, Minneapolis 1, Minn.
AMPHETAMINES:
George McClellan, Food and Drug Administration, Baltimore 2, Md.
ASPIRIN AND PHENOBARBITAL:
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SPECTROPHOTOMETRIC METHODS—INFRARED:
J. Carol, Food and Drug Administration, Washington 25, D. C.
SPECTROPHOTOMETRIC METHODS—ULTRAVIOLET:

SULFONAMIDE DERIVATIVES:
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STEROID ESTROGENS:
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SYNTHETIC ESTROGENS:
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GLYCOLS AND RELATED COMPOUNDS:

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MICROSCOPIC TESTS FOR ALKALOIDS AND SYNTHETICS:
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ORGANIC IODIDES AND SEPARATION OF HALOGENS:
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PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS:

COSMETICS:
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John E. Clements, Food and Drug Administration, Washington 25, D. C.
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C. F. Bruening, Food and Drug Administration, Chicago 7, Ill.
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S. H. Newburger, Food and Drug Administration, Washington 25, D. C.
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Referee: K. A. Freeman, Food and Drug Administration, Washington 25, D. C.
ARSENIC AND ANTIMONY IN COAL-TAR COLORS:
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INORGANIC SALTS IN COAL-TAR COLORS:
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NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
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PAPER CHROMATOGRAPHY OF COAL-TAR COLORS:
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VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS:
K. S. Heine, Jr.

SUBCOMMITTEE C:

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

PROCESSED VEGETABLE PRODUCTS:

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

COFFEE AND TEA:

Referee: ———
CHLOROGENIC ACID IN COFFEE:
L. C. Weiss, Food and Drug Administration, Los Angeles 15, Calif.
MOISTURE IN COFFEE AND TEA:
B. Krinitz, Food and Drug Administration, New York 14, N. Y.

DAIRY PRODUCTS:

Referee: Wm. Horwitz, Food and Drug Administration, Washington 25, D. C.
CRYOSCOPY OF MILK:
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FAT IN DAIRY PRODUCTS:
Ernest O. Herreid, Dept. Food Technology, University of Illinois, Urbana, Ill.
FAT IN HOMOGENIZED MILK:
Claude E. Hynds, Department of Agriculture and Markets, Albany 1, N. Y.
FOREIGN FATS IN DAIRY PRODUCTS (CHROMATOGRAPHIC PROCEDURES):
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FOREIGN FATS IN DAIRY PRODUCTS (REICHERT-MEISSL DETERMINATION):
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FROZEN DESSERTS:
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PHOSPHATASE TEST IN DAIRY PRODUCTS:

PREPARATION OF BUTTER SAMPLES:
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RECONSTITUTED MILK:
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SAMPLING, AND PREPARATION OF SAMPLE, OF SOFT CHEESES:
Sam Perlmutter, Food and Drug Administration, Minneapolis 1, Minn.
SAMPLING, FAT, AND MOISTURE IN HARD CHEESES:
Wm. Horwitz

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

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES):
Referee: W. I. Patterson, Food and Drug Administration, Washington 25, D. C.
ANIMAL FECAL MATTER:
John Thomas, Food and Drug Administration, New Orleans 16, La.
APPLES:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
FISH (HISTAMINE):
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FISH PRODUCTS (ACIDS):
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LACTIC ACID:

PINEAPPLE (DECOMPOSITION, CARBOHYDRATE):
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SHELLFISH:
Helen Barry, Food and Drug Administration, New Orleans 16, La.
SPINACH (SUCCINIC ACID):
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STRAWBERRIES:

TOMATOES (SUCCINIC ACID):
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URIC ACID IN CEREAL PRODUCTS:
Helen Barry
URIC ACID IN NUTS:
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GELATINE, DESSERT PREPARATIONS, AND MIXES:
Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.

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

FISH AND OTHER MARINE PRODUCTS:

Referee: Menno D. Voth, Food and Drug Administration, Seattle 4, Wash.
SALT AND SOLIDS IN OYSTERS:
John P. Traynor, Food and Drug Administration, Baltimore 2, Md.
TOTAL SOLIDS AND ETHER EXTRACT IN FISH AND MARINE PRODUCTS:
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GUMS IN FOODS:

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

CATSUP AND RELATED TOMATO PRODUCTS:
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CHEESE SPREADS:
Raymond H. Johnson, Food and Drug Administration, Seattle 4, Wash.
DRESSINGS FOR FOODS:
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FROZEN DESSERTS:
J. Thomas Welch, Food and Drug Administration, Baltimore 2, Md.

MEAT AND MEAT PRODUCTS:

Referee: Roger M. Mehurin, Meat Inspection Div., Bur. of Animal Industry, Washington 25, D. C.
CHEMICAL TESTS FOR IDENTIFICATION OF MEATS:
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CREATIN IN MEAT PRODUCTS:
J. M. McCoy, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.
LACTOSE:

MOISTURE AND FAT IN MEAT PRODUCTS:
Ernest Windham, Vet. Division, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C.
SEROLOGICAL TESTS FOR IDENTIFICATION OF MEATS:
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STARCH IN MEAT PRODUCTS:
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METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS:

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ALDRIN:
Felix Sabatino, Food and Drug Administration, Washington 25, D. C.
BENZENE HEXACHLORIDE:
A. K. Klein, Food and Drug Administration, Washington 25, D. C.
CHLORDANE AND HEPTACHLOR:

COPPER:

DDT AS SPRAY RESIDUE ON FOODS:
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DIELDRIN:

FLUORINE:
P. A. Clifford, Food and Drug Administration, Washington 25, D. C.
INSECTICIDES IN CANNED FOODS:
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MERCURY:
A. K. Klein
METHOXYCHLOR:
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PARATHION:
P. A. Clifford
SODIUM:
P. A. Clifford
SODIUM FLUOROACETATE (1080):
L. L. Ramsey
ZINC:
O. R. Alexander, American Can Company, Maywood, Ill.

MICROBIOLOGICAL METHODS:
Referee: M. T. Bartram, Food and Drug Administration, Washington 25, D. C.
EGGS AND EGG PRODUCTS:
M. T. Bartram

MICROCHEMICAL METHODS:
Referee: C. L. Ogg, Eastern Regional Research Lab., Philadelphia 18, Pa.
ELEMENTAL ANALYSIS:
C. L. Ogg
GROUP ANALYSIS:
A. Steyermark, Hoffman-LaRoche, Inc., Nutley, N. J.

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

SHREDDED COCONUT (GLYCOLS AND GLYCEROL):
J. C. M. Griffin, Food and Drug Administration, Atlanta 3, Ga.
STARCH IN PEANUT BUTTER:
G. L. Patrick, Food and Drug Administration, Atlanta 3, Ga.

OILS, FATS, AND WAXES:
Referee: Gardner Kirsten, Food and Drug Administration, New York 14, N. Y.
ANTIOXIDANTS:
S. Kahan, Food and Drug Administration, New York 14, N. Y.
COLOR IN OLEOMARGARINE:
Louis L. Gershman, Food and Drug Administration, New York 14, N. Y.
PEANUT OIL:
A. B. Karasz, Dept. of Agriculture and Markets, Albany 1, N. Y.
SPECTROPHOTOMETRIC METHODS:
David Firestone, Food and Drug Administration, New York 14, N. Y.

SPICES AND OTHER CONDIMENTS:

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

PREPARATION OF SAMPLE OF FRENCH DRESSING:

A. F. Ratay, Food and Drug Administration, Philadelphia 6, Pa.

SEEDS AND STEMS IN GROUND CHILI:

SORBITOL:

Felice A. Rotondaro, Food and Drug Administration, Philadelphia 6, Pa.

SUGAR, ASH, AND PUNGENT PRINCIPLES IN MUSTARDS:

Jesse E. Roe, Food and Drug Administration, Denver 2, Colo.

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ENZYMES:

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ENZYMIC METHODS FOR INSECTICIDES:

J. W. Cook

SUBCOMMITTEE D:

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

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PRESIDENT'S ADDRESS

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION AND THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

By HARRY J. FISHER (The Connecticut Agricultural Experiment Station, New Haven 4, Conn.)

It has been the custom since the earliest days of our Association for the president to deliver a presidential address. Of late years these addresses have usually been of a historical nature. Since the founding of the Connecticut Agricultural Experiment Station in 1875, its Department of Analytical Chemistry (of which I have the honor to be the head) has had six men in charge, and all of these men became presidents of the A.O.A.C. The subject of my paper was therefore almost foreordained. Its preparation has been a fascinating study, and I hope that I can make the subject interesting or at least not boring to you.

The Connecticut Agricultural Experiment Station is the oldest agricultural experiment station in the United States; it celebrated its 75th anniversary in 1950. As I stated, during its 78 years of existence it has had six heads of its chemistry department, all of whom became presidents of the Association of Official Agricultural Chemists. It is about these men and some of their associates at the Station, the parts they played in the advancement of the fields of chemistry in which we are all interested—and particularly about their influence on this Association and how it in turn helped them—that I wish to talk this afternoon.

For the sake of convenience this paper will be divided into six sections, each relating primarily to one of the department heads, but discussing also some of their contemporaries who played important parts in this Association even though they never presided over it.

SAMUEL WILLIAM JOHNSON

Samuel W. Johnson was the first president of the Association of Official Agricultural Chemists; he was also active in the earlier meetings of agricultural chemists that led to the foundation of our Association at Philadelphia in 1884.

Probably to Johnson more than to any other man is due the credit for introducing the idea of agricultural experiment stations to the United States. He was born at Kingsboro, New York, in 1830, became interested in chemistry at an early age, had his own private laboratory at 18, and published an article on the analysis of limestone when he was only 19. From the first, his particular interests concerned the application of

chemistry to agriculture; these interests became confirmed by his studies in 1850 under J. P. Norton at the then new Yale School of Applied Chemistry and later under Erdmann and Liebig in Germany. He returned from Europe impressed with the value to agriculture of the experiment stations he had seen there and determined to work toward the establishment in this country of similar stations for the benefit of the American farmer. He accepted a teaching position on the Yale faculty which he never relinquished, but at the same time he acted as chemist for the State Agricultural Society in analyzing fertilizers and lectured constantly on the benefits that would be derived from a state agricultural experiment station. In 1875 this campaign bore fruit, although not exactly in the form that Johnson had intended, when the state legislature appropriated $700.00 a quarter for two years for the establishment of an agricultural experiment station at Wesleyan University in Middletown—the first such station in the United States.

Johnson was not director of the Connecticut Agricultural Experiment Station for the two years it was at Middletown; that position was filled by the then professor of chemistry of Wesleyan University, W. O. Atwater, who was later to be in charge of the Office of Experiment Stations of the U. S. Department of Agriculture and to become famous for his work on the caloric value of foods. (In passing it should be noted that Professor Atwater also played a part in this Association and in the earlier meetings of agricultural chemists that led to the formation of the A.O.A.C.)

In 1877 the Connecticut Agricultural Experiment Station was transferred to New Haven and put on a permanent basis under the directorship of Professor Johnson. Three years later Johnson began his connection with what was to become the A.O.A.C.

I have not time to go into all the details of the meetings that led to the organization of the A.O.A.C. Those who are interested should consult Dr. Harvey Wiley's very full account which was published in Bulletin 57 of the old Division of Chemistry of the U. S. Department of Agriculture. It all began with a circular letter that J. T. Henderson, then Commissioner of Agriculture of Georgia, sent to commissioners of agriculture, state chemists, and professors of chemistry in state universities and agricultural colleges on May 20, 1880, asking them to attend a convention for the purpose of establishing uniform methods for the analysis of fertilizers. This convention was held in Washington on July 28, 1880, and was followed by other meetings in Boston and Cincinnati that were held in connection with meetings of the American Association for the Advancement of Science. Uniform methods for the determination of phosphoric acid, nitrogen, and potash were drawn up by committees, tentatively agreed upon, and later found not to be entirely satisfactory (particularly the method for citrate-soluble phosphoric acid). Steps were then taken to organize as a subdivision of the A.A.A.S.

E. H. Jenkins of the Connecticut Agricultural Experiment Station took an active part in the first Washington meeting; both Johnson and Atwater were present at the Boston meeting, where Johnson was appointed chairman of a committee of five " . . . to secure the cooperation and experimental research of agricultural chemists, to collect and examine the various published methods of fertilizer analysis. . . . " Only Atwater was present from Connecticut at the Cincinnati meeting.

After the meeting in Cincinnati in 1881, a feeling developed among the official chemists that they should have an association in which they could reach decisions of their own, uninfluenced by the commercial chemists. This was brought to a head by another call from Judge Henderson for a meeting in Atlanta on May 15, 1884; this meeting led to another at Philadelphia at the time of the A.A.A.S. meeting on September 8 and 9, 1884, at which the Association of Official Agricultural Chemists was born. E. H. Jenkins of Connecticut presided at this meeting, at which Johnson was elected as the first president of the A.O.A.C.

Although Johnson had attended the earlier Atlanta meeting with Jenkins, he was not in Philadelphia when he was elected, and failed to attend the second convention in Washington the following year. In fact, it was not until the thirteenth convention in 1896 that the first president of the A.O.A.C. attended one of its meetings for the first and only time. Nevertheless, he had taken an active part in preparing the way for the founding of the Association, and supported it through the years until his retirement as director of the Experiment Station in 1900 by encouraging his chemists to contribute generously of their time to its studies.

I would not want to give the impression that Johnson's only contributions—or even his most important ones—were as an administrator. First of all he was a teacher, a pioneer in what at that time was the new field of agricultural chemistry, and many agricultural students first learned the scientific facts of agriculture from his two books, *How Crops Grow* and *How Crops Feed.* He was also a practicing analytical chemist who was adept at devising new pieces of apparatus; up to this very year of 1953 the Connecticut Agricultural Experiment Station laboratory was using apparatus bearing his name to determine fat in feeds, and a bank of Kjeldahl stills, built in New Haven under his direction in the 1880's, was still in use two years ago—and doing as good a job as the "Labconco" apparatus that replaced it.

There were other members of the Station staff of the early days who were later to play a part in Association affairs after they left Connecticut. When the Station first opened in New Haven in 1877 Johnson's original staff consisted of himself, E. H. Jenkins, and H. P. Armsby. Of Jenkins I shall speak presently; Armsby later became Professor of Agricultural Chemistry at Wisconsin and then Director of the Pennsylvania Agricultural Experiment Station; he took part in the 1892 and 1896 meetings of

the Association. In 1883 E. H. Farrington joined the staff; he later specialized in the chemistry of dairy products and eventually became chief of the Dairy Department at the University of Wisconsin. Farrington attended many of the earlier meetings of the Association; at the fourth convention in 1887 Jenkins and he presented reports on the determination of moisture, fat, and "albuminoids" in milk; by the fifth meeting he had transferred to New Hampshire and attended as a representative of that State; the tenth convention in 1893 saw him present from Illinois, and the next year he read the report on dairy products.

Charles D. Woods, who had been on the staff during its first two years in Middletown and had remained at Wesleyan University with Atwater, represented Connecticut at the seventh meeting of the Association in 1890 and was a regular attendant for some years thereafter. W. J. Jordan (who later became director of the Maine Agricultural Experiment Station) and Milton Whitney (later chief of the Bureau of Soils of the U. S. Department of Agriculture) were others.

EDWARD HOPKINS JENKINS

Edward H. Jenkins, the third president of the A.O.A.C., was elected to that position at the third convention in Washington on August 26–27, 1886.

Jenkins was born at Falmouth, Massachusetts, on May 31, 1850. He graduated from Yale College in 1872 and returned to the Sheffield Scientific School to prepare himself for teaching. His exposure to Johnson at "Sheff" awakened his interest in agricultural chemistry, and after studying at Leipzig and Tharandt in 1875 he returned home in 1876 and joined the staff of the newly organized Connecticut Agricultural Experiment Station at Middletown. He was the only one of the original staff who followed the Station to New Haven when it was transferred there in 1877, and he remained at the Station until his semi-retirement as Director Emeritus in 1923. He thus served the Station and the State 47 years on a full-time basis, and for the eight remaining years of his life he was a regular visitor to the Station, always willing to help the lowliest member of the staff who asked his advice.

Jenkins was in a sense an active member of our Association even before the Association was founded, because he took part in the various agricultural chemists' meetings between 1880 and 1884 that led up to the organization of the A.O.A.C. at Philadelphia in 1884. He was a regular attendant at meetings for many years, and always played an important rôle in the Association's affairs.

There were no A.O.A.C. referees during the first years; instead the various subjects (mostly in the fertilizer field) were assigned to committees. Jenkins presented the report of the Committee on Phosphoric

Acid in Fertilizers at the second convention of the Association in 1885, citing experiments carried on in his laboratory to compare the relative efficacy of hydrochloric and nitric acids as solvents for phosphates. At the same meeting he shared membership on the Committee on Nitrogen in Fertilizers with Philip Chazal and Harvey Wiley of later pure food fame. At the following (third) meeting he was elected to serve as president for the ensuing year. At the fourth convention in 1887, besides presiding, he and Farrington gave several reports on the determination of water, fat, and "albuminoids" in milk. At the eighth convention in 1891 he brought with him from New Haven both A. W. Ogden and Andrew Winton; eventually he was to relinquish to Winton regular attendance at meetings of the Association, but he still came to meetings until the twentieth convention at Washington in 1903. When at the 1897 meeting the Association decided to appoint a committee "to consider standards of purity of the foods and drinks on sale in the United States", Jenkins was made a member of this first Standards Committee along with Wiley, Scovell, Weber, and Frear, and remained on this committee for several years. At the sixteenth meeting at San Francisco in 1899 (the only meeting of our Association ever held on the West Coast) he was appointed to the Committee on Recommendations of Referees—a committee that had been established for the first time a year before.

Jenkins's earliest interests had lain in teaching; therefore it was perhaps natural that, while he performed his analytical duties ably and conscientiously, he interested himself from his first years with the Station in getting out into the field, meeting farmers, and talking over their problems with them. His talents both as a speaker and as an administrator were so obvious that he was foreordained to succeed Johnson when the latter retired as director in 1900.

Jenkins was still in active charge of the Station when I first came to work there in October, 1921, and I well remember our first meeting. An impressive, goateed figure wandered into the laboratory, stood behind me watching me weigh out feed samples, and presently engaged me in a discussion of the relative accuracy of mineral and feed analyses. By the time he left he had somehow communicated to me the feeling that I was part of one family, all working together and all helping each other. That was how he ran the Station and why he was so good as an administrator.

ANDREW LINCOLN WINTON

Winton was elected president of the A.O.A.C. at its fourteenth convention in 1897 and presided at the fifteenth meeting the following year. He was thus its fourteenth president.

Andrew Winton was born at Westport, Connecticut, on January 26, 1864. He graduated from the Sheffield Scientific School of Yale University

in 1884 (where he was a student of Johnson), and the following September went to work as a chemist at the Connecticut Agricultural Experiment Station.

Winton's connection with our Association began with his attendance at the eighth convention in 1891 and continued uninterruptedly, even after his retirement, almost up to the day of his death in 1946; in fact it was only shortly before he died that he wrote a friend of the delight with which he was looking forward to seeing his old acquaintances once again at the meeting that year.

At the first meeting he attended—in 1891—he presented a paper "On the Use of Sodium Chloride in the Lindo-Gladding Method of Determining Potash", and two years later he was Reporter on Dairy Products (the title "Referee" had not yet been adopted) and gave a paper on "The Babcock Gravimetric Method for the Determination of Solids in Fat in Milk—(Drying with Asbestos in a Perforated Metal Cylinder)". In 1895 he was Associate Reporter on Potash in Fertilizers and was made a full Reporter for the following year. At the 1896 Convention he was elected vice-president, and the following year was elected president for 1897–1898. The proceedings of the 1897 meeting also refer to him as "Referee on Potash"; this is the first use of the term "Referee" that I have discovered. In his presidential address in 1898 he discussed the changing pattern of the Association's work, pointing out that "During the first two years of its existence our organization was really a body of fertilizer analysts, as only methods for the analysis of these materials were considered", while by 1886 fodders and dairy products were being referred to committees, and by 1896 "all articles used for food or drink" were being taken up.

Winton's active part in referee work by no means ceased when he had served out his term as president. In 1897, as I pointed out before, the Association had appointed a committee to draw up food standards; to Winton was assigned the task of making the analyses of authentic samples of spices that were required before standards for spices could be set. The report of the Standards Committee in 1898 recorded that in the one year of his appointment Winton had already sampled more than 100 lots of original imports of spices and was busy analyzing them and studying them microscopically; by 1900 the committee reported that he had furnished them the results of 206 analyses of spices and their adulterants, together with descriptions of the microscopic and chemical methods he had used. At that 1900 meeting Winton was appointed Associate Referee both on spices and condiments and on baking powders; in 1901 he gave the report of Committee B on Recommendations of Referees (the system of appointing subcommittees to examine and report on the work of referees began in that year); and in 1902 he became Associate Referee on Flavoring Extracts. He remained Associate Referee on Spices for a number of years, even after transferring from State to Federal service in

1906. He served as a member of the committee which prepared one of the pre-first editions of our "Methods", the *Official and Provisional Methods of Analysis*, issued as Bureau of Chemistry Bulletin 107 in 1907.

Winton had left the Connecticut Agricultural Experiment Station fifteen years before I came there; I met him much later at A.O.A.C. meetings when he was an old man, but I never really knew him and can judge of him as a person only from what others have said. While he was a pioneer in food chemistry, undoubtedly his most important contribution to knowledge was in the field of the microscopy of foods. He became interested in this subject soon after joining the Station staff, and in 1899 he went to Europe to study microscopic technic under Professor Moeller at Graz. His book, *Microscopy of Vegetable Foods*, published in 1906, is still considered an authority on the subject, and the later four-volume, *Structure and Composition of Foods*, that he and his second wife published jointly between 1932 and 1939 is a constant source of reference for everyone interested in the composition of foods.

During the later years Winton was at the Station he and his assistant Kate Barber worked long hours preparing microscopic sections of spices and other foodstuffs for his forthcoming publications. On May 1, 1907 he resigned from the Station staff to become chief of the Chicago Food and Drug Laboratory of the Bureau of Chemistry of the U.S.D.A.; shortly thereafter Dr. Barber left the Station to enter Federal service in Washington, and in 1911 they were married.

In 1909, when Albert Leach of the Massachusetts State Department of Health became ill, Winton took over (purely as an act of friendship) the editing of the second edition of Leach's *Food Inspection and Analysis*, and after Leach died in 1910 Winton was solely responsible for both the third edition and the fourth and last edition, which, although it appeared as long ago as 1920, is still the first book to be consulted by all food analysts.

Winton had always been an enthusiastic supporter of Harvey Wiley in his pure food campaign, and when Wiley resigned as Chief of the Bureau of Chemistry in 1912 Winton submitted his own resignation two years later. The Wintons then set up a private consulting laboratory in Wilton, Connecticut, but continued to attend A.O.A.C. meetings. In 1936 he gave the Wiley Memorial Address.

JOHN PHILLIPS STREET

Street was elected president of the A.O.A.C. at its twenty-third convention held at Washington on November 14–16, 1906, and presided at the following meeting in 1907. He was the twenty-third president of our Association.

John Phillips Street was born at Beverly, New Jersey, on January 30, 1869. After graduation from Rutgers he joined the staff of the New Jersey

Agricultural Experiment Station, where he remained until he accepted the appointment to replace Winton as head of the chemistry department of the Connecticut Agricultural Experiment Station in 1907.

Street's introduction to the A.O.A.C. was his attendance at the seventh convention in Washington in 1892; from then on he hardly missed a meeting until he left State service on returning from the army in 1918. At the 1892 meeting he read a paper on "The Ulsch Method in Determining Nitric Nitrogen in Complete Fertilizers", and in 1895 he presented a report on a comparison of the use of two different magnesia mixtures for the determination of phosphoric acid in fertilizers. At the 1896 meeting he was Reporter on Nitrogen in Fertilizers and described an Ulsch-Street method for nitrate nitrogen that was adopted by the Association as official in 1897. In 1898, when a Committee on Recommendations of Referees was appointed for the first time, Street became a member of that committee together with Huston and Ross. At the 1906 meeting he was elected president for the ensuing year, was made Associate Referee on Feeds, and gave a paper on "The Detection of Peat in Commercial Fertilizers" which outlined a method of estimating the amounts of peat in fertilizers from their pentosan contents.

The year that elapsed between Street's election as president and his presiding at a meeting coincided with his transfer from the New Jersey to the Connecticut Station, so he actually came to the Connecticut Agricultural Experiment Station as president of the A.O.A.C. The 1907 meeting at which he presided was one of the few held outside of Washington; it took place at Norfolk, Virginia, in connection with the Jamestown Exposition.

Street continued to serve the A.O.A.C. in various capacities so long as he remained in State service. He was Associate Referee on Vegetables and a member of Subcommittee A from 1909 to 1910, and chairman of Subcommittee A from 1910 to 1914; at the 1910 meeting he gave a paper (jointly with C. B. Morison) on "Ginger Extract".

While Street's work at New Jersey had been largely concerned with fertilizers, when he transferred to Connecticut he became deeply interested in the many patent medicines then being so promiscuously sold with fantastic curative claims. Under his direction, the laboratory of the Connecticut Agricultural Experiment Station analyzed many of these proprietaries, and he himself compiled all available information from other sources on the composition of such "remedies". The combined results were published by him in a book, *The Composition of Patent and Proprietary Medicines*, that is still consulted.

When World War I started, Street obtained a leave of absence to enter military service, and went to France to take charge of an Army food laboratory. He was retired as a major at the end of the war, and returned to the Connecticut Agricultural Experiment Station only to resign to

organize inspection work for the National Canners' Association at Indianapolis. Three years later he went to Rochester, New York, as executive secretary of the New York State Canners' Association, a post he retained until his death on September 22, 1938.

Because Street did not attend A.O.A.C. meetings for the last twenty years of his life he was unknown to most of us here today. Besides Johnson he was the only one of my predecessors at the Station whom I never met.

EDWARD MONROE BAILEY

With Bailey, we approach the more modern days of our Association; he was well known to many of you here. He was elected president in 1929 and presided at the 1930 meeting.

Edward Monroe Bailey was born on August 27, 1879 at New London, Connecticut, but spent most of his early life on a farm at Higganum. After graduation from high school and a short experience as a country schoolteacher, he entered Sheffield Scientific School of Yale University, from which he was graduated in 1902. Within a month of his graduation (in July, 1902) he joined the staff of the Connecticut Agricultural Experiment Station; there he remained until his retirement 43 years later in 1945.

Bailey's first contact with the A.O.A.C. came when he attended the twenty-first convention at St. Louis in 1904. (Incidentally this was also the first meeting at which Julius Hortvet of Minnesota, known to all milk analysts for his invention of the Hortvet cryoscope, was present.) Bailey was immediately thrown into the thick of A.O.A.C. activities by his appointment as Associate Referee on Fruit Products, and the following year he presented a report on a method for the determination of moisture in dried fruits. At this same 1905 meeting he was made Associate Referee on Cocoa and Cocoa Products. Later he was Referee on Dairy Products (1913), Tea (1918 and 1919), Cacao Products (1924 and 1926), and Coffee (1932 and 1933); his last refereeship appointments were as Associate Referee on Alcohol in Drugs in 1928 and as Associate Referee on Colorimetric Methods for Vitamins the following year. He served as Chairman of the Committee on Recommendations of Referees continuously from 1926 to 1934. Perhaps the most important of all his contributions to the Association was his undertaking the responsibility, as chairman of the Committee on Editing *Methods of Analysis*, of preparing the fourth and fifth editions of our book of methods.

As chairman of the Committee to Confer with the American Public Health Association on Standard Methods of Milk Analysis from 1932 to 1943, he was responsible for the chemical sections of several editions of the A.P.H.A. *Standard Methods of Milk Analysis*. He served on the Advisory Food Standards Committee under the old Wiley Law from 1924 to 1940.

I came to work under Bailey in 1921, and in the 24 remaining years before he retired in 1945 we were together every day. I can therefore speak of him from a first-hand knowledge that I did not have of any of his predecessors. Of his character I can say that I never knew anyone who had an ill word to say of him or who did not hold him in the utmost respect. He never tolerated slipshod work or mental dishonesty from his subordinates, but he was always ready to offer them sympathy and help when they needed it.

Bailey came to be regarded as an expert on the composition of foods. When Street left the Station for army service in 1918, Bailey took his place and remained in charge of the department of analytical chemistry for the next 27 years. During his headship of the department the laboratory specialized in the analysis of the many special foods for diabetics that were on the market before the advent of insulin. Compilations of analyses of both these foods and common foods prepared by Bailey were published in Station bulletins that still remain in demand; these tables were reproduced in several editions of Joslin's *Treatment of Diabetes Mellitus*.

Bailey published several papers on his own investigations into the chemistry of foods and drugs: studies on the composition of the banana; methods for the analysis of noodles; the determination of vanillin, coumarin, and acetanilid in imitation vanilla extracts; the detection of sulfites and the determination of starch in meat; tests for the anthraquinone drugs, etc.

HARRY JOHNSTONE FISHER

We all have seen in cemeteries monuments bearing a person's name and birth date with the date of death left blank. I have no desire to emulate such persons and write here this afternoon the beginning of my obituary. But since this paper is intended to outline the connections between the Connecticut Agricultural Experiment Station and the Association through the years, and to express my pride that the Association has honored all six of its chemistry department heads with election to the presidency, I do wish to speak very briefly of my own connection with the A.O.A.C.

I attended my first A.O.A.C. meeting in 1926, coming there on my own from the American Legion Convention in Philadelphia. What I remember chiefly of that meeting is our filing by President Coolidge to get a limp handshake, and an oyster roast somewhere on the banks of the Potomac at which almost no one could open the oysters and we ended up by eating frankforts. At that time I was not an important enough member of the Station staff to be sent to Washington at State expense, and it was not until the thirties that I became a regular attendant and held several associate refereeships on drug topics. Then in 1934 the Association honored

me by appointing me to Subcommittee B; my third term on that committee will end next year.

It was no doubt because of my experience in assisting Bailey in his editorial work on the fourth and fifth editions of *Methods of Analysis* and the training I had received from him that I was honored by being appointed chairman of the Committee on Revision of Methods for the sixth and seventh editions. I do have what I think is a legitimate pride that the institution for which I work should have been entrusted with the responsibility of preparing the last four editions of *Methods of Analysis.*

This paper would be incomplete if I did not mention others still at the Station who have worked actively for the Association. Dr. Rebecca Hubbell, who has been a regular attendant for years, has been active in the vitamin section and in one of our ancillary organizations, the Animal Nutrition Research Council. Mr. Mathis is Referee on Emission Spectrographic Methods and Mr. Richard Merwin is Associate Referee on Drugs in Feeds. Some years ago Dr. Hubert Vickery, head of our biochemistry department, served as Associate Referee on free ammonia in plants and wrote for our Journal a paper on the history of the Kjeldahl method; and very recently Mr. Lloyd Keirstead of my laboratory held an Associate Refereeship on oil emulsions. Others have served frequently as collaborators, and I can assure you that in the future the Station will continue to be ready to serve the Association in any way it can.

I hope that in this recital of some of the facts of the 73-year connection between the Connecticut Agricultural Experiment Station and the Association of Official Agricultural Chemists I have succeeded in conveying to you some of the interest that I feel, and have not bored you. If Johnson and Jenkins were here today they would find much that was familiar to them, but they would be amazed at the size of our meetings (there were 11 present at the first meeting in Philadelphia in 1884) and at the series of meetings of related Associations stretching on into the rest of the week. If it is permitted to a retiring president to offer counsel before he bows out, I would like to say just one more thing. The specialized control officials' associations that have grown up through the years—the Association of American Feed Control Officials, the Association of American Fertilizer Control Officials, and the Association of Economic Poisons Control Officials—have all remained faithful to and looked up to the A.O.A.C. as the mother organization and have resisted occasional ill-advised efforts of a few of their members to have them go separate ways. May I hope that the A.O.A.C. itself will continue to realize that these associations all perform a function that our Association has no wish to assume but which is nevertheless important—namely, the promotion of uniformity in law enforcement—and that we will continue to cherish them as our children.

ADDRESS TO THE WOMEN CHEMISTS OF THE A.O.A.C.*

By Mrs. Anna Kelton Wiley

It is an honor and a pleasure to bring a greeting to the women chemists of the Association of Official Agricultural Chemists. It is an honor, because I feel that it is a token of respect to Doctor Wiley, in whose life the A.O.A.C. played such a large part. And it is a pleasure, because I have devoted my life to the forward-going of women and rejoice to see so many women chemists gathered together. It was not so long ago that there was no such thing as a woman chemist. I have tried to find out when the first women chemists "invaded the ranks" of the A.O.A.C. but have been unable to do so.

Doctor Wiley always enjoyed his association with the A.O.A.C. and was one of its founders. He was its president in 1886 and served as its secretary from 1884 to 1912, when he retired from public service. One of my most delightful memories is that of October 18, 1924 (the fortieth anniversary of the A.O.A.C. and the eightieth anniversary of his birth) when, at a dinner here in Washington, he was presented with a handsome bronze plaque on which was depicted his likeness, name, and degrees. At each place was a smaller replica of the plaque, and on its back was another engraving which depicted the caduceus of the physician, the retort of the chemist, maize for the farmer, and an oak cluster for old age and the pure food law, accompanied by the words: "In recognition of his service to chemistry, agriculture, hygiene, and the public welfare." You will be interested to know that I have had this medallion carved on his tombstone in Arlington.

A pamphlet printed by the Association of Official Agricultural Chemists at the time of his death, entitled "In Memoriam—Harvey Washington Wiley," is one of the sweetest tributes to him which I possess.† It contains statements about Doctor Wiley's life by W. W. Skinner, who said: "We are met with the sad realization that a silver thread binding the events of the Association is broken," and ended by quoting the great Agassiz, Doctor Wiley's instructor at Harvard:

"Come, wander with me
In regions yet untrod,
And read what is still unread
In the manuscript of God."

Other tributes in the book are from C. A. Brown, Walter G. Campbell,

* Presented at the luncheon meeting of women chemists of the A.O.A.C. held at the Shorham Hotel, Washington, D. C., Oct. 13, 1953.
† See *This Journal*, 14, iii (1931).

A. S. Mitchell, W. D. Bigelow, and H. A. Huston. Mr. Huston, an old friend who is here today, said in his speech on "Wiley—The Organizer of the A.O.A.C." that "the factor of greatest weight in winning the appointment as Chief Chemist in the U. S. Department of Agriculture was his reputation as an authority on the production and analysis of sugars." He spoke of Doctor Wiley as a great organizing secretary, and said: "As the Association year after year added subject upon subject to its activities, Doctor Wiley could always be depended on to give most valuable and cordial support to every new project. The growth in size of the printed *Methods of Analysis* from a four-page folder to a closely printed volume of more than five hundred pages serves to indicate in one way the magnitude of the work of the central figure in the development of the Association." Affectionate tributes from Mary Tidd Read, whom I knew very well, and from Fred B. Linton, for forty years Chief Clerk of the Bureau of Chemistry, complete a dearly prized book.

In preparing this greeting, I learned that the A.O.A.C. was started in Philadelphia in the year 1884. I found that a few of our leading agricultural chemists met in July, 1880 in Washington, D. C., to devise ways and means for the improvement of analytical methods. The outcome of this and three successive meetings was the formation of the A.O.A.C. which began its career in the Utopian Club of Philadelphia in 1884. The purpose was to "secure as far as possible uniformity in legislation with regard to the regulation of the sale of commercial fertilizers in the different states and uniformity and accuracy in the methods and results of fertilizer analysis."

So, sixty-nine years ago your origin as an association had primarily to do with the soil and its fertility.

In a poem which Doctor Wiley wrote on this first meeting, he said:

"In a dingy hall in Philadelphia,
Seeking a cool retreat
Where, free from the heat of the parchéd town
Utopians drink and eat,

A few of the faithful counsel held
Over K and N and P,
While the deepening shadows fell,
Was born the A.O.A.C.

Great was the shout of joy that rang
From the throats of the faithful few,
And many a time said throats were laved
With cool Gambrinian dew.

Jenkins the jolly sat at the head;
Richardson, Myers, and White,

Dabney, Wiley, Chazal, *et al.*,
Were ranged to left and right.*

Deep were the plans for the future laid,
The draughts they drank were deep,
The sausages are all consumed,
But memories will keep!" . . . and so on.

Doctor Wiley, son of a farmer, was born in 1844 near the banks of the Ohio River in Indiana. He worked in the soil from the age of eleven. He earned his food and room rent, while at Hanover College, by going home each Saturday and tilling his father's farm. He fought in the Civil War, but by 1870 had earned the degrees of A.B. and A.M. at Hanover College. Subsequently he was Professor of Chemistry at Indiana Medical College, 1872–1874; Professor of Chemistry at Butler University, 1874; Professor of Chemistry at Purdue, and State Chemist of Indiana, 1874–1883. He also went abroad and studied chemistry under celebrated chemists in Berlin. He won a B.S. in chemistry in six months of work at Harvard in 1872–1873, the only man ever to do so. So his early life was full of agriculture and chemistry and it is no wonder that his first work was "Principles and Practice of Agricultural Analysis; A Manual for the Study of Soils, Fertilizers, and Agricultural Products."

In the preface to the first edition, which came out in 1895, he wrote:

> "In this volume I have endeavored to place in the hands of teachers and students of agricultural analysis, and of analysts generally, the principles which underlie the science and art of the analysis of soils and the best approved methods of conducting it."

The second edition came out in 1906 and in the preface Doctor Wiley states that the advancement of agricultural science had been so rapid he found it necessary to rewrite practically the entire book.

The third edition was published in 1926 and in the preface he says:

> "As I write this preface I look back upon the first and second editions as historical events and look forward to the fourth edition in the spirit of prophecy."

He also tells that after the completion of the manuscript he offered it to several publishers, but because of its magnitude and lack of general interest, no publishing firm would undertake the task of printing it. Finally he undertook to publish it himself. Dr. Edward Hart, president of the Chemical Publishing Company, an old friend of ours, finally offered to continue the publication. No one anticipated that there would be a large demand for a book of this kind; nevertheless, the demand has been steady through all these years. Dr. Wiley gave all the rights to this work

* Edward Hopkins Jenkins, Clifford Richardson, John A. Myers, Henry Clay White, Charles Wm. Dabney, H. W. Wiley, and P. E. Chazal.

to the A.O.A.C. before his death in 1930 when he wrote: "I have full faith in the foster parents of my child . . . I take this occasion to say farewell to my many friends in all parts of the civilized world who have made use of this book."

A year ago, the Library of Congress requested and received all of Doctor Wiley's papers and records, for which I was very happy; however, I still have some souvenirs of his extraordinary life of hard work and achievement.

Here is one of his whimsical poems "Dedicated to Mary Tidd Read, or if she declines, to Bertie Trescott," two of the earliest members of the old Bureau of Chemistry and old friends of mine.

"Review of Dr. Wiley's *Principles and Practice of Agricultural Analysis, Vol. I.*"

"There was an old government fake
Who bethought him some money to make,
So he got up a book of the extracts he took
From the works of some chemical rake;
And this old government crook
To pad out the size of his book
Did call on his friends
For such odds and ends
As would give it a most learnéd look.

He got out a volume on soil
That represented considerable toil,
Of a kind which a mule
That walked past a school,
Would consider good cause for turmoil.
He filled it with methods galore,
From foreign parts culled by the score,
Of the kind known of late
As quite out of date,
And busted the record for sure."

. . . . . .

In reviewing the proceedings of your Association from the Sixth to the Nineteenth Convention, I was impressed with the part it played in the expansion of agricultural chemistry and its influence on the passage of the federal food and drug act of 1906, so popularly known as "Dr. Wiley's Pure Food Law." One has only to read the presidential addresses during those years to get an appreciation of the contributions made by the A.O.A.C. I have in mind particularly those of John A. Myers, 1889; H. A. Huston, 1895; B. B. Ross, 1896; Wm. Frear, 1897; A. L. Winton, 1898; B. W. Kilgore, 1900; and L. L. Van Slyke, 1901. Of particular significance

are the remarks of Dr. Frear and Dr. Kilgore. The former said: "The prime work of this Association is along lines important to official agricultural chemists . . . but parallel with fertilizer work there has risen a demand upon agricultural chemists . . . for the detection of food adulteration, with a view to insuring its prevention." Dr. Kilgore, in 1900, said: "The Association has been called upon to aid in the education of public sentiment and to bring influence to bear in favor of a national pure food bill." The Association was the first to recognize the need for standards for foods. Its first committee on the subject consisted of Wm. Frear, H. W. Wiley, H. A. Weber, M. A. Scoville, and H. A. Jenkins. They instituted the formulation of food standards which is now an established legal function under the Federal Food, Drug and Cosmetic Act of 1938.

Unfortunately, or maybe it is fortunate for you as well as for myself, I did not have all the Annual Reports of the A.O.A.C. to consult. Had I all sixty-nine reports, I probably would not be here today to give this greeting. But I have read enough to convince myself what a wonderful group you are and how proud I am to have been the wife of one of its founders and how proud you women chemists must be to belong to it.

Many tributes have been paid to Dr. Wiley for his untiring work and unflagging zeal in behalf of the origin, development, and passage of the Pure Food Law of 1906. His hard and often bitter fight for the interests of the consumer against the then prevailing spirit of *caveat emptor* is too long and perhaps too well known to be recounted here. I would like to quote from A. L. Winton's speech on the occasion of the Memorial Symposium on Food, sponsored by the Division of Agricultural and Food Chemistry of the American Chemical Society, on September 12, 1944 to celebrate the one hundredth anniversary of Dr. Wiley's birth. He said:

> "Although great indeed was Dr. Wiley's service in securing for all Americans purer and better foods, his lasting fame rests on the impetus he gave to food chemistry in the interests of agriculture, nutrition, food manufacture, and food economy. The number of chemists in the field of food chemistry has grown from less than a dozen in a rented tenement to many thousands in spacious laboratories throughout the land. And so I say, feelingly and gratefully, that Dr. Wiley, successively linguist, chemist, physician, sociologist, and journalist, was the outstanding pioneer in food chemistry and food research, and may well be heralded as the Father of American Food Chemistry."

At the Commemorative Fortieth Anniversary of the passage of the Federal Food and Drugs Act, held under the auspices of the Section on Food, Drug, and Cosmetic Law of the New York State Bar Association on June 25, 1946, Mr. Charles Wesley Dunn, Chairman of the New York State Bar Association's Section on Foods, listed the 103 bills which pre-

ceded the passage of the final Act. He gave the A.O.A.C. much credit for the basic study of methods for food analysis and, to my great satisfaction, he stated: " . . . in reviewing such action, we will show that Dr. Harvey W. Wiley was the founder of this Act."

In closing, let me call to your attention the fact that everything that has been said or quoted in this greeting which I have brought you emphasizes the truth in the poem by John Greenleaf Whittier, who wrote:

"Yet sometimes glimpses on my sight,
Through present wrong, the eternal right;
And step by step, since time began,
I see the steady gain of man."

WEDNESDAY—MORNING SESSION

REPORT OF THE EDITORIAL BOARD

WILLIAM HORWITZ, *Chairman*

Inasmuch as a Committee on Revision of *Official Methods of Analysis* was not appointed during the past year because of the difficulty in finding a chairman, such a committee must be appointed during the coming year to begin the necessary editorial work. A problem which will eventually require some action by the Association involves the increase in size of *The Journal.* The increased editorial load of *The Journal,* a cumulative index, and the forthcoming revision of *Official Methods of Analysis* has resulted in the employment of a full time rather than a part time assistant editor.

REPORT OF THE EDITORIAL COMMITTEE OF *THE JOURNAL*

PAUL A. CLIFFORD, *Editor and Chairman*

The total number of *Journal* pages for 1953 will be over 1,200 (the largest volume to date). The August number alone comprised 467 pages. Contributed papers and notes totaled 70, in contrast to 47 for Volume 35 (1952).

These evidences of growth are, of course, gratifying. It has become apparent that over a period of years, *The Journal* has gradually evolved from a simple record of Association proceedings into a publication of considerable scientific status. The increasing number of contributed papers on a wide variety of scientific topics is witness to this fact.

We welcome these contributions in spite of the expense of printing them; *The Journal* has always operated at a financial loss. Costs of printing alone will come to over $17,000 this year.

It has been our custom to publish the annual reports of Referees and Associate Referees in the May and August numbers of *The Journal* in the year following their presentation. As a result, the May and August numbers of *The Journal* are oversized and usually tardy in appearing. It may be necessary to equalize the size of our four numbers, perhaps by incorporating some of the reports into the February number. The necessity for this might become acute if *all* Referees and their Associates reported on their assigned topics. For instance, this year reports were due on 277 subjects; reports were given on only 151. The percentage of Ref-

erees reporting this year, 54.5 per cent, appears about normal (percentages reporting in 1952 and 1951 were 51.5 and 50 per cent respectively). Although it would be extremely desirable for all Referees to report, such action would greatly increase the size of *The Journal* with a resultant increase in expense and editorial work.

Perhaps more of the contributed papers could be reserved for the November number. This might be an unwelcome alternative, as we are eager to give our contributors as early publication as possible.

However, these growing pains are merely evidence of progress and increase in our prestige.

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

The Tenth Edition of *Standard Methods for the Examination of Dairy Products*, by the American Public Health Association, is scheduled for printing next month [November, 1953.] Directions are arranged similarly as in the previous edition. Major changes include: (1) Substitution of milk-free plating media for rehydrated media to which one per cent of skim milk was added before plating; (2) substitution of phosphate-buffered distilled water for tap water in preparing serial dilutions of samples; (3) a warning about the use of ion-exchange treated waters as substitutes for distilled water in media rehydration, dilutions, etc.; and (4) deletion of methods for the determination of vitamins and for extraneous matter in dairy products, exclusive of sediment in fluid milk. These deletions were made because the methods were rarely used in public health laboratories.

For the first time directions have been given for: (1) The detection of antibiotics in fluid milk and, in the case of penicillin, for its identification; (2) the detection of such microbic growth inhibitors as the quaternary ammonium compounds; (3) the milk ring test for brucellosis in herd milk containing milk from one or more infected animals; (4) an improved copper sulfate modification of Scharer's phosphatase test; (5) a modified Babcock method for fat in homogenized milk; and (6) a complete description of the A.O.A.C. method for residual phosphatase in cheese. Use of a semi-automatic syringe to replace the calibrated loop is described for transferring test portions of 0.01 ml when milk and cream films are prepared for the direct microscopic method. A coliform standard has been proposed for frozen dairy foods and for such fermented milk drinks as cultured buttermilk. To assist administrators, directions for interpreting

the results of positive coliform tests and explaining their causes have been clarified.

Methods for sediment in milk now include use of both a coarse and a fine sediment mixture for preparing standard comparison discs. Details of procedures for thermoduric and thermophilic species have been stated more systematically. Because of the trends for two- and three-day shipments of milk and cream to consuming areas (for the use of cold wall tanks on farms and for every-other-day retail deliveries), increasing attention has been given to the importance of psychrophilic bacteria as an index of recontamination and/or bacterial growth following pasteurization.

The new edition contains not only general directions for collecting representative samples from large storage and/or transportation tanks and vats, but also presents practical procedures whereby it can be determined whether the method of sampling assures representation.

The American Public Health Association and the Committee take this opportunity to acknowledge the courtesies extended by the Association of Official Agricultural Chemists in permitting the inclusion in the Tenth Edition of chemical methods selected from the Seventh Edition of *Official Methods of Analysis*, for the analysis of dairy products and to express gratitude for the timely help given by individual members of your Association.

WM. HORWITZ
F. LEE MICKLE
A. H. ROBERTSON, *Chairman*

REPORT OF A.O.A.C. COMMITTEE TO CONFER WITH A.S.T.M. ON SOIL CONDITIONERS

During the Sixty-sixth Annual Meeting of the Association of Official Agricultural Chemists, an informal round table discussion was held on the general subject of soil conditioners. The meeting was attended by representatives of several manufacturers or distributors of soil conditioners and a number of state and Federal officials. At this meeting the industry made clear its desire that methods be devised for the testing of soil conditioners for performance or for effectiveness when added to soils. Some of the control officials pointed out that methods of this type were not commonly used in law-enforcement work and that nearly all of the official methods were based on the composition or on the physical or chemical properties of the product distributed. The industry representatives expressed little hope that suitable methods based on composition could be devised. They indicated that the American Society for Testing Materials

had been contacted and that efforts were being made to work out a program for the development of methods.

In a letter dated October 13, 1952, Mr. L. C. Gilbert, Assistant Technical Secretary of the A.S.T.M., wrote to H. A. Lepper, Secretary-Treasurer of the A.O.A.C., that its Board of Directors, at the September meeting, had authorized the organization of a new Technical Committee on Soil Conditioners. Mr. Gilbert asked if the A.O.A.C. wished to participate at a meeting to be held in Philadelphia on November 7, 1952. President Fisher appointed the General Referee on Fertilizers to attend this meeting for the A.O.A.C. and charged him with the responsibility for organizing work on soil conditioners during the current year.

The meeting in Philadelphia was attended by the following persons:

R. E. Hess, Associate Secretary and Editor-in-Chief, A.S.T.M.
L. C. Gilbert, Assistant Technical Secretary, A.S.T.M.
P. D. Cauldis, California Packing Company.
L. S. Hitchner, National Agricultural Chemicals Association.
H. A. Hashbarger, Monsanto Chemical Company.
C. J. Mighton, E. I. du Pont de Nemours.
W. P. Martin, Ohio State University.
L. T. Alexander, U. S. Department of Agriculture.
S. J. Toth, Rutgers University.
F. W. Quackenbush, Purdue University.

Dr. Martin acted as temporary chairman. Representatives from industry pointed out the need for testing methods and promulgation of standards which would eliminate ineffective soil conditioners from the market. Members of the A.S.T.M. indicated interest in conditioners for soil stabilization or other uses of an engineering character, and it was stated that the A.O.A.C. intended to go ahead with methodology relating to testing and analysis of soil conditioners sold or used for agricultural purposes. There seemed to be a considerable area of mutual interest and it was agreed that officers of the A.O.A.C., A.S.T.M., and the Soil Science Society of America should be asked if they wish to participate in a joint committee to develop a program on soil conditioners.

President Fisher appointed the following committee to make recommendations as to whether the A.O.A.C. should participate in a joint committee with the A.S.T.M. and other groups:

F. W. Quackenbush, General Referee on Fertilizers, *Chairman*.
W. H. MacIntire, General Referee on Soils and Liming Materials.
R. Q. Parks (later C. S. Slater), U. S. Department of Agriculture.

This committee recommended participation of the A.O.A.C. in such a joint committee, with the understanding that none of the methods, nomenclature, or standards which might be developed must necessarily be adopted by the A.O.A.C. This recommendation was approved by the

A.O.A.C. Executive Committee. Officers of the Soil Science Society declined official representation on the joint committee.

An organizational meeting of the joint committee was held in Columbus, Ohio, on September 3, 1953. Invitations were issued to the following:

PRODUCERS (12)

American Chemical Paint Company (M)*.....Mr. Beatty
American Cyanamid Company (M).....Dr. F. L. Stark
Du Pont de Nemours & Company (M).....Mr. C. J. Mighton
General Aniline & Film Corp. (M).....Mr. J. W. Lang
Wyandotte Chemicals Corp.....C. F. Gerlach (M)
Goodrich Chemical Co. (M).....Mr. H. N. Stevens
Hercules Powder Company (M).....Mr. L. H. Burt
Monsanto Chemical Co. (M).....
.....Mr. H. A. Hashbarger and J. C. Engibous
National Agricultural Chemicals Assn.....Mr. L. S. Hitchner
Rohm & Haas Co. (M).....Mr. R. P. Hopkins
American Polymer Corp.....Leonard Shapiro (M)
Union Carbide & Carbon Corp. (M).....Mr. K. L. Smith

CONSUMERS (6)

California Packing Corp.....Mr. P. D. Caldis
Campbell Soup Co.....Dr. Eldrow Reeve
H. J. Heinz Company.....Mr. C. A. John
O. M. Scott and Sons Co.....Mr. Victor Renner
Seabrook Farms, Inc.....Dr. Frank App
Sears, Roebuck & Co. (M).....Dr. W. F. Spikes

GENERAL INTEREST (23)

R. E. Hess and L. C. Gilbert.....A.S.T.M. Officers
H. J. Fisher, *President* and W. Horwitz, *Secretary*.....
.....A.O.A.C. Officers
John Carter.....Battelle Memorial Institute (M)
Miles D. Catton, Portland Cement Association, and K. B. Hough, Cornell University. Committee D-18, Soils for Engineering Purposes
W. T. Lambe.....Massachusetts Institute of Technology (M)
L. T. Alexander and C. S. Slater..U. S. Department of Agriculture
Bert Lexen.....U. S. Forest Service Research
F. N. Briggs.....University of California
N. T. Coleman.....North Carolina State College
W. H. Daniel.....Purdue University
A. E. Erickson.....Michigan State College
W. H. Fuller.....University of Arizona

* (M) Signifies Member of the A.S.T.M.

W. H. Gardner.....University of Washington
R. J. Hervey.....Texas Agricultural Experiment Station
Donald Kirkham.....Iowa State College
W. H. MacIntire.....University of Tennessee
J. P. Martin.....Citrus Experiment Station, California
W. P. Martin.....Ohio State University
R. D. Miller.....Cornell University
F. W. Quackenbush.....Purdue University
W. A. Raney.....Mississippi State College
L. A. Richards.....University of California
M. B. Russell.....University of Illinois
C. L. W. Swanson....Connecticut Agricultural Experiment Station
G. S. Taylor.....Ohio State University
S. A. Taylor.....Utah State Agriculture College
S. J. Toth.....Rutgers University
K. B. Woods (M).....Purdue University

A large majority of those listed attended. The following statement of the scope of the committee's activities was agreed upon:

"The formulation of specifications and methods of testing of materials for the alteration of physical properties of soil for agricultural and engineering purposes; the selection of acceptable nomenclature and the stimulation of research to accomplish the foregoing purposes."

Officers of the joint committee were elected for one year as follows:

Chairman.....W. P. Martin
Vice-Chairman.....W. A. Raney
Secretary.....H. M. Stevens

It was agreed that these officers, together with the subcommittee chairmen, three persons to be named by the A.O.A.C., and three to be named by the A.S.T.M. would constitute an Executive Committee for the joint committee.

The discussion indicated that the following three subcommittees should be formed at the earliest possible time:

(*a*) Nomenclature.
(*b*) Methods of testing for performance.
(*c*) Methods of testing for composition and properties.

The next meeting of the Executive Committee was scheduled for mid-November at Dallas, Texas, during the meetings of the Soil Science Society.

The Referee recommended that, during the year, the A.O.A.C. appoint two Associate Referees on soil conditioners, the first (preferably a member

of the Soil Science Society) to be concerned with methods of evaluating performance of these products when added to the soil, and the second to be concerned with methods of analysis to determine composition and purity. Dr. S. J. Toth of Rutgers University was appointed Associate Referee on Performance.

A questionnaire on soil conditioners sent to all state control laboratories brought replies from 38 as follows:

21 considered that current practices of distribution presented a serious problem.
5 stated that their present state laws covered soil conditioners.
1 anticipated passage of a law by current legislature to cover these products.
2 had initiated work to develop methods of analysis.
3 would accept appointment as Associate Referee on analysis for composition.
2 would accept appointment as Associate Referee on performance.
8 were willing to spend some time to develop methods.
29 would collaborate on methods developed in other laboratories.
9 would be unable to help on methods.

RECOMMENDATIONS

It is recommended that——

(1) A General Referee on soil conditioners be appointed.

(2) An Associate Referee on methods of testing for composition and properties be appointed at the earliest possible time.

(3) The joint committee with the A.S.T.M. be continued and that three A.O.A.C. representatives be named to its Executive Committee by the Association.

F. W. QUACKENBUSH, *Chairman*
W. H. MACINTIRE
C. S. SLATER

Approved.

PRELIMINARY REPORT OF THE A.O.A.C. COMMITTEE ON SPECTROPHOTOMETRY

The Committee on Spectrophotometry met on June 5, 1953, and on October 12, 1953, in Washington, D. C. It was decided that the Committee would submit a preliminary report to the Association at the 1953 meeting; this report was to cover: (*a*) Proposed Nomenclature; and (*b*) Methods of Presenting Spectrophotometric Data (Curves). It is em-

phasized that this is a preliminary report, and that the recommendations are subject to revision and improvement. Comments are invited from persons interested in this subject.

NOMENCLATURE

The Committee agreed that in so far as nomenclature is concerned, the most important consideration is the standardization of terms. The advantages of the use of a standardized nomenclature in the publications of the Association are obvious. It appears equally desirable to have the nomenclature adopted by this Association agree as nearly as possible with that used in other scientific publications dealing with applied spectroscopy. For this reason, it was felt desirable to have our recommendations agree, wherever possible, with the recommendations of the Joint Committee on Nomenclature in Applied Spectroscopy [*Anal. Chem.*, **24**, 1349 (1952)]. The terms recommended in that report have been widely adopted by other workers in the field of spectroscopy.

The discussions of the Committee brought out the fact that the only terms over which there has been any substantial disagreement as to nomenclature and definition were the terms *transmittance*, *absorbance*, and *Beer's Law*. After some discussion, the Committee agreed to recommend the use of the terms *transmittance* and *absorbance* to secure uniformity with the Joint Committee report.

In our opinion the definitions of *transmittance* and *absorbance* given in the Joint Committee report are not completely satisfactory, particularly when applied to solutions. We have, therefore, recommended somewhat different definitions which we believe more adequately define the terms used in solution spectrophotometry. Our definition of *Beer's Law* is simply another way of stating the conditions implied in the "absorption laws"; it is believed, however, that this is the form in which the law is most frequently used.

Most of the other proposed definitions are essentially the same as those given in the Joint Committee report. Minor changes have been made in some of the definitions to make them agree more nearly with the usages of solution spectrophotometry.

It is suggested that for terms not specifically mentioned in this report the recommendations of the Joint Committee should be followed.

SUGGESTED NOMENCLATURE*

Absorbance(s) (*A*).—The negative logarithm to the base 10 of the ratio of the transmittance of the sample to that of the reference or standard material.

* The definitions of the terms indicated by an (*) were adopted without change from Report No. 6 of the Joint Committee on Nomenclature in Applied Spectroscopy; *Anal. Chem.* **24**, 1349 (1952).

$$A = -\log_{10} T/T_0 = \log_{10} (1/T) - \log_{10} (1/T_0).$$

This definition implies that the reference must be fully described for the term to have a specific meaning. For solutions, the reference is usually (but not necessarily) the solvent in the same or equal cell. The reference used should always be stated (see *Presentation of Data*).

Other names that have been used for the quantity represented by this term are *optical density, extinction,* and *absorbancy.*

Absorption band.—A region of the absorption spectrum in which the absorptivity passes through a maximum.

Absorption peak (maximum).—A point on the curve of absorptivity (or absorbance) versus wavelength where the slope is zero and changes from positive to negative as wavelength increases. At this point the absorptivity is a maximum and the wavelength is a critical value.

Absorption minimum.—A point on the curve of absorptivity (or absorbance) versus wavelength where the slope is zero and changes from negative to positive as wavelength increases. At this point the absorptivity is a minimum and the wavelength is a critical value.

Absorptivity(ies) (a).—The absorbance per unit concentration and cell length. $a = A/bc$, where b is in centimeters and c in grams per liter, or $a = (A/bc) \times 1000$ if c is in milligrams per liter.

Other names that have been used for this quantity (or related quantities) are *extinction coefficient, specific absorption, absorbance index,* and $E^{1\%}_{1cm}$.

Absorptivity, Molar (ϵ).— $\epsilon = a \times$ mol. wt.

Angstrom (A).—A unit of length equal to 10^{-8} centimeter or 10^{-10} meter.

Beer's Law.—The absorptivity of a substance is a constant with respect to changes in concentration and/or cell length, or $A = abc$.

Cell length (b).—The internal length in centimeters of the cell used.

Concentration (c).—The quantity of the substance contained in a unit quantity of the sample. The preferred unit for ultraviolet and visible work is milligrams per liter.

*Determination.**—The ascertainment of the quantity or concentration of a specific substance in a sample.

Infrared.—The region of the electromagnetic spectrum from ca 0.78 micron to 300 microns.

*Light.**—Radiant energy in the spectral range visible to the human eye (ca 3800–7800 *A*).

*Micron** (μ).—A unit of length equal to 10^{-6} meter or 10^{-4} centimeter.

*Millimicron** (mμ).—A unit of length equal to one thousandth of a micron.

*Photometer.**—An instrument for measuring relative radiant power or relative luminous flux or some function of these quantities.

*Radiant power** (P).—The rate at which energy is transported in a beam of radiant energy.

Spectrophotometric.—Of or pertaining to spectrophotometry.

Spectrophotometry.—The branch of spectroscopy dealing with the measurement of relative radiant power or relative luminous flux as a function of wavelength or frequency.

*Spectroscopy.**—The branch of physical science which treats of spectra.

*Standard Sample.**—A material of known composition which clearly resembles in chemical and physical nature the materials with which the analyst expects to deal and which is employed for calibration.

Standard Sample, Primary.—A standard sample whose composition is certified by a recognized standardizing agency or group.

*Stray Radiation.**—All radiation which reaches the detector at wavelengths which do not correspond to the spectral position under consideration.

Transmittance(s), (T).—The ratio of the radiant power transmitted by the sample to the radiant power incident on the sample, when both are measured at the same spectral position and with the same slit width. The beam is understood to be parallel radiation and incident at right angles to plane parallel surfaces of the sample.

If the sample is a solution the solute transmittance is the quantity usually desired and is determined directly as the ratio of the transmittance of the solution in a cell to the transmittance of the solvent in an equal cell. This quantity has often been called transmittancy.

The quantity represented by this term has also been called transmission.

Ultraviolet.—The region of the electromagnetic spectrum from about 100 to 3800 A. The term ultraviolet without further qualification usually means the region from ca 2000 to 3800 A.

*Visible.**—Radiant energy which is perceived by the normal eye (approximately 3800–7800 A).

*Wavelength** (λ).—The wavelength is the distance, measured along the line of propagation, between two points which are in phase on adjacent waves. The value in a vacuum may be symbolized: λ_{vac}.

*Wavenumber** (ν).—The number of waves per unit length in a vacuum; the reciprocal of λ_{vac}. The usual unit of wavenumber is the reciprocal centimeter, cm^{-1}.

PRESENTATION OF SPECTROPHOTOMETRIC DATA

The Committee agreed that greater uniformity in the presentation of spectrophotometric data was desirable. The following rules are recommended:

The instrument used in obtaining the data should be clearly speci-

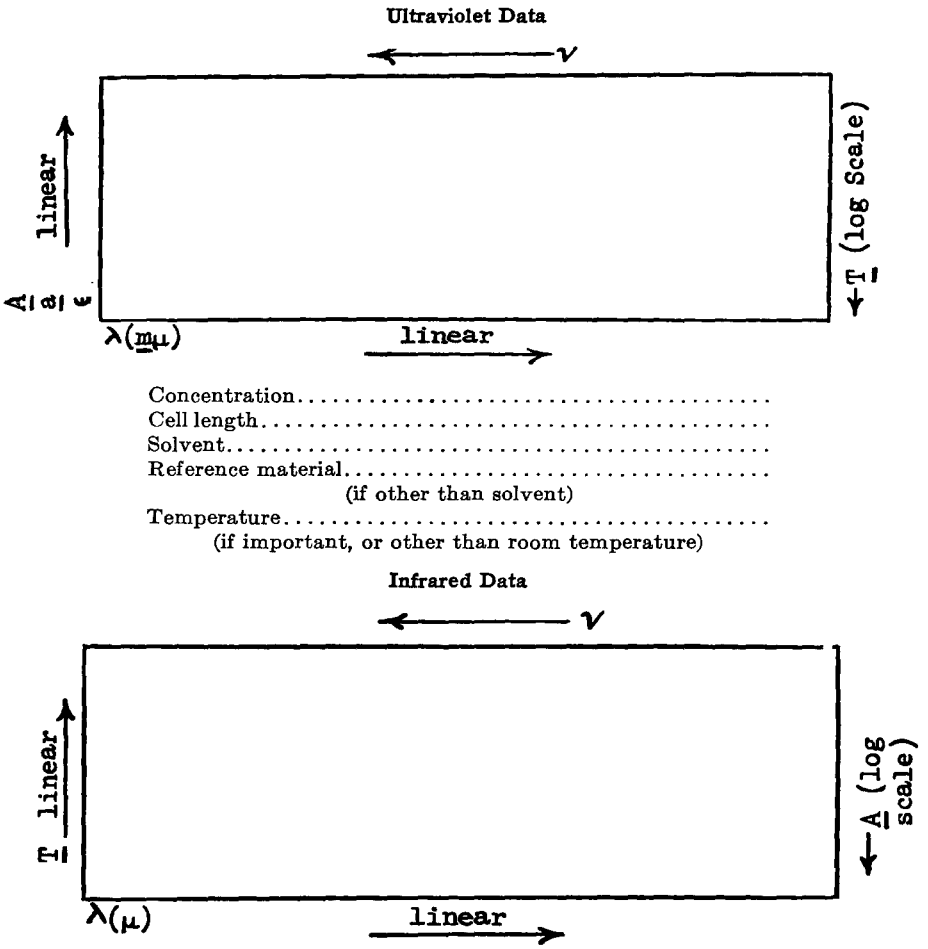

FIG. 1.—Proposed methods of presenting ultraviolet and infrared data.

fied, including, where pertinent, the source, slit width, monochromator, phototube, etc.

The following data should be given for each spectrophotometric curve presented for publication:

(a) The solvent, if any, used for the sample.

(b) The reference material, particularly if it differs in any way from the solvent.

(c) The concentration of the solution used to determine the spectral data.

(d) The cell length.

(e) The temperature, if other than room temperature.

Ultraviolet and visible absorption curves for publication should conform to the following rules (see Fig. 1):

(a) The wavelength should be plotted as a linear function on the abscissa.

(b) The wavelength should increase from the left to the right.

(c) The wavelength should be given in millimicrons.

(d) The ordinate should be linear in A, a, or ϵ. [Plots of A (actual data) are preferable.]

(e) The data are plotted with the higher values of A at the top of the plot.

(f) Transmittance values, if indicated, should be on a logarithmic scale corresponding to the proposed absorbance scale.

(g) Frequencies, if shown, should be on a reciprocal scale corresponding to the suggested wavelength scale.

(h) Other methods of plotting should be considered only if necessary for better comprehension of the data.

Infrared absorption curves should conform to the following rules (see Fig. 1):

(a) The wavelength should be plotted as a linear function on the abscissa.

(b) The wavelength should increase from left to right.

(c) The wavelength should be given in microns.

(d) The ordinate should be linear in transmittance.

(e) Increasing values of transmittance are plotted upward.

(f) Absorbance values should be on a logarithmic scale, corresponding to the proposed transmittance scale.

(g) Frequency values, if shown, should correspond to the proposed wavelength scale.

The suggestion has been made that the next (1955) Edition of *Official Methods of Analysis* should contain a chapter on Spectroscopy. Such a chapter might contain generalized procedures for spectrographic, spectrophotometric, and flame photometry methods of analysis, and would eliminate the need for repetition of similar instructions throughout the book. Comments on the inclusion of such a section in *Official Methods of Analysis* are invited. The Committee agreed to assist in the preparation of such a chapter if the Editorial Board feels it desirable.

B. A. Brice
Jonas Carol
W. T. Mathis
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John H. Jones, *Chairman*

Approved.

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

WM. F. REINDOLLAR, *Chairman*

The 1953 Annual Meeting of the A.O.A.C. is being held approximately two weeks later than those in recent years, affording additional time for the submission of reports and for their consideration by the several subcommittees. The usual letters of instruction and notification were sent out by the Chairman during the past year and the response by Referees and Associate Referees has been deemed generally satisfactory. Definite progress is noted on many of the assigned topics.

The difficulty of securing competent analysts to serve as Referees, Associate Referees, and collaborators is a continuing one and markedly handicaps the Association programs. It must be realized that advances can be made only in proportion to the number of those willing to accept an assignment and participate in this important work.

Once again the Chairman wishes to acknowledge the valuable contributions made by the subcommittees, Referees, Associate Referees, and collaborators, and to express his sincere appreciation to them. It is through the efforts of these groups, extending over the years, that the publication of *Official Methods of Analysis* has been made possible.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

J. B. SMITH (Rhode Island State College, Kingston, R. I.), *Chairman;* C. V. MARSHALL; and C. D. TOLLE

FEEDING STUFFS

It is recommended—

(1) That the study of ash and of milk by-products in feeds be discontinued.

(2) That work on the following be continued:

Crude fat or ether extract.
Crude protein in feeding stuffs.
Drugs in feeds.
Fat in fish meal.
Microscopic examination.
Mineral constituents of mixed feeds.

(3) That Associate Referees be appointed for:

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

Molasses in feeds.
Gossypol in feeds.

(4) That the method for crude fat in baked dog food adopted first action last year be revised as recommended by the Associate Referee and remain first action.

(5) That the method for crude protein in feeds **(22.10)** be changed to read, "Det. N as under **2.24** using mercury as the preferable catalyst in case of material digestible with difficulty. Multiply results by 6.25, or in the case of wheat grains, by 5.70."

(6) That the method for Enheptin ® (2-amino-5-nitrothiazole) proposed by the Associate Referee be adopted, first action.

(7) That the method for *p*-arsanilic acid, as modified with respect to sample weight and volume of solvent subsequent to collaborative study, be adopted, first action.

(8) That the method for total solids in highly acid milk by-products in mixed feeds, recommended by the Associate Referee, be adopted, first action, and that the Committee on Revision of *Official Methods of Analysis* consider publication of this procedure with other methods for total solids in the chapter on Dairy Products.

FERTILIZERS

It is recommended—

(1) That investigation of methods for the direct determination of available P_2O_5 in fertilizers be continued, and that the use of perchloric acid in the preparation of phosphate fertilizer solutions be studied.

(2) That the interference of minor elements in methods for potash and the use of the flame photometer for that determination be studied further.

(3) That collaborative study of methods for boron be continued and that a procedure for active water-insoluble boron be devised.

(4) That methods for collection, storage, and transfer of aliquots of samples of ammoniacal liquids be studied further.

(5) That the method for water-soluble magnesium in coarse particles presented in 1952 by the Associate Referee be changed to prescribe a sieve with openings of 420 microns and be adopted, first action.

(6) That methods for sampling and sample preparation be studied further.

(7) That all the Referees continue their work during the coming year.

ECONOMIC POISONS

It is recommended—

(1) That the following editorial comment be added to method **5.142–5.143** (Chloramine T): after "Cl" in the last line add, "To convert active Cl to available Cl, multiply active Cl by 2."

(2) That the revisions in method **5.112** (Pyrethrin II), *This Journal*, **35**, 64 (1952), adopted first action, be made official.

(3) That method **5.113–5.114** (Pyrethrum Extracts in Mineral Oil) adopted as first action, be made official.

(4) That the study of analytical methods for the determination of pyrethrins, including the hydrochloric acid modification of the official mercury reduction method, be continued.

(5) That methods for the determination of rotenone, dieldrin, aldrin, piperonyl butoxide, phenolic disinfectants, dithiocarbamates, and systemic insecticides be further studied.

(6) That the proposed modification of method **5.149** (gamma isomer-BHC) be studied collaboratively.

(7) That the O'Keefe and Averell method for technical parathion, parathion dusts, and emulsifiable concentrates, as modified by the Associate Referee, be subjected to further collaborative study.

(8) That collaborative studies of methods for the determination of physical properties of insecticidal powders be initiated.

(9) That the study of the modified Eble method for warfarin be continued.

(10) That methods for the determination of volatility hazards of hormone-type herbicides be studied collaboratively.

(11) That methods **5.21, 5.22, 5.23,** and **5.24** (fluorine), **5.124** (sulfur) and **5.8, 5.9, 5.37** (arsenic), now first action, be made official.

(12) That the method for total chlorine in esters of 2,4-D and 2,4,5-T in liquid herbicides by the Parr bomb–boric acid procedure, *This Journal,* **36,** 367, 379 (1953), adopted as first action, be made official.

(13) That the name of this chapter be changed from "Economic Poisons" to "Pesticides."

(14) That methods for higher boiling phenols be studied further with special attention to those of petroleum origin.

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

DISINFECTANTS

It is recommended—

(1) That study of the "Use-dilution" methods for evaluating disinfectants be continued.

(2) That an Associate Referee be appointed to investigate the sporicidal test proposed by Ortenzio, Stuart, and Friedl, *This Journal,* **36,** 480 (1953).

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

(4) That method **5.166** (Fungicidal Test) be amended as recommended by the Associate Referee and remain first action.

PLANTS

It is recommended—

(1) That the study of methods for sodium in plant materials be continued with special attention to solvents for its extraction, and to flame photometric procedures.

(2) That methods for sodium in plants be studied collaboratively.

(3) That the anthrone procedure for starch in plant materials be studied further.

(4) That the ion-exchange resin procedure for clarification of plant extracts containing sugars as described by the Associate Referee, *This Journal*, **36**, 401 (1953) be adopted, first action, for addition to the method for sugars, **6.48.**

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

STANDARD SOLUTIONS

It is recommended—

(1) That the method for standardization of sodium thiosulfate solutions be studied further.

(2) That the modification of the official method **39.11** for constant boiling hydrochloric acid, *This Journal*, **36**, 354 (1953), adopted as first action, be changed by the substitution of the equations suggested by the Associate Referee for the calculation of the air weight to give one equivalent weight, and that the modified method remain first action.

(3) That the study of constant boiling hydrochloric acid be discontinued.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That study of methods for soil *p*H be continued.

(2) That work on the method for potassium using the flame photometer be continued.

(3) That the correlation of exchangeable hydrogen values indicated by the official calcium acetate procedure, with calcium sorption and *p*H values on different types of soils, be studied further.

(4) That the colorimetric determination of molybdenum be studied collaboratively, as recommended by the Associate Referee.

(5) That automatic titration devices for the determination of fluorine be studied collaboratively.

(6) That studies of the "Combination dithizone-spectographic method" and of the polarographic procedure for zinc be continued.

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

(8) That the use of carmine and "Chromotrope B" as indicators of the boron content of soils be studied further.

(9) That the survey of methods for the determination of phosphorus in the "available" state and the ratio of organic to inorganic phosphorus, *This Journal*, **30**, 43 (1949), be studied further.

(10) That the Associate Referee on calcium and magnesium in soils continue his work.

NUTRITIONAL ADJUNCTS

It is recommended—

(1) That the status of the method for vitamin A in mixed feeds be continued first action, and that a saponification procedure be subjected to further collaborative study.

(2) That the study of the determination of vitamin A in margarine along the lines indicated in this year's report be continued.

(3) That the work on carotene be continued.

(4) That studies on analysis for xanthophyll in mixed feed be continued, and that collaborative samples be submitted for analysis by a chromatographic method.

(5) That the short method as applied to thiamine in enriched flour be adopted as first action and that the study be continued to consider other enriched cereal products.

(6) That the chemical method for nicotinic acid have the following statement added as a footnote:

"For the extn of materials contg bran, after autoclaving with 1 *N* H_2SO_4 adjust the mixt. to ca pH 13 by addn of 10 *N* NaOH and allow to stand at room temp. for 15 min. Then adjust the pH to ca pH 4.5 by addn of (3+1) HCl and proceed as usual. For the extn of glutenous material with ca 1 ounce samples, use 400 ml 1 *N* H_2SO_4."

(7) That work be continued to develop a chemical method for vitamin B_6.

(8) That work be continued on the development of a satisfactory microbiological method for the determination of pantothentic acid in natural materials.

(9) That the method studied this year by the Associate Referee for the assay of vitamin B_{12} in materials containing approximately 0.1 micrograms or more of vitamin B_{12} per gram or per ml (0.05 milligram per pound or more) be adopted, first action, replacing the first action method for the assay of vitamin B_{12} feed supplements containing from approximately 1.0 milligram to 10 milligrams vitamin B_{12} per pound, which was adopted last year, *This Journal*, **36**, 96 (1953).

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

(11) That all Associate Referees continue study of their topics.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

HARRY J. FISHER (Agricultural Experiment Station, New Haven, Connecticut), *Chairman;* G. ROBERT CLARK, and JONAS CAROL

RADIOACTIVITY

No report was received. The Committee recommends that the subject be continued.

EMISSION SPECTROGRAPHY

The Referee points out that because no two spectrographic laboratories working with agricultural materials have identical equipment, it is not feasible to adopt spectrographic methods that rigorously specify all details of apparatus and technic. He states that collaborative analyses of the same samples by different laboratories, each using its own equipment, have nevertheless shown that it is possible to prescribe certain procedures with specified permissible variations that will yield satisfactory results. He recommends the adoption as first action of two methods, using respectively DC arc and AC spark excitation, which he describes.

The Committee concurs in the recommendation of the Referee.

VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended—

(1) That because the Associate Referee's report on aminophylline and phenobarbital does not show which collaborators used predetermined "*A*" values, and because the spread in results is rather wide, the proposed method involving solvent extraction and spectrophotometric determination be studied another year. The Committee recommends that in the future all reports involving spectrophotometry be accompanied by curves covering the particular portion of the spectrum involved.

(2) That study of the method for the separation of quinine and strychnine, outlined in the report of the Associate Referee for 1952, be continued in the manner suggested by the Referee.

(3) That the subject of aminopyrine, ephedrine, and phenobarbital be reassigned.

SYNTHETIC DRUGS

It is recommended—

(1) That the study of the chromatographic separation of acetophenetidin and caffeine be continued.

(2) That the recommendations of the Associate Referee and Referee that the study of the determination of Propadrine hydrochloride in vari-

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

ous vehicles be continued, and that the name of the subject be changed to phenylpropanolamine hydrochloride, be approved.

(3) That the first action method for benzedrine **(32.25)** be dropped and that the following methods for amphetamine outlined in the Associate Referee's report be adopted as first action:

(a) The titrimetric determination of total amphetamine.

(b) The confirmatory gravimetric determination of total amphetamine as the acetyl derivative.

(c) The determination of stereochemical composition of amphetamine by polarimetric examination of the acetyl derivatives.

(d) The confirmatory determination of stereochemical composition by thermal analysis of the acetyl derivative.

It is also recommended that the subject of amphetamines be continued for the purpose of investigating ultraviolet absorption methods.

(4) That the study of methods for the separation of phenobarbital and aspirin be continued.

(5) That the methods for the determination of sulfadiazine in the presence of other sulfonamides, and of sulfamerazine and sulfadiazine in mixtures of these two drugs, outlined by the Associate Referee, be adopted as first action. It is also recommended that methods for the determination of sulfa drugs by titration in organic solvents be submitted to collaborative study.

(6) That, because the Associate Referee has shown that the present first action gravimetric method for ketosteroids, *This Journal*, **34**, 81 (1951), may yield high results, the text of the method for ketosteroids be revised in the manner outlined by the Associate Referee.

(7) That the subject of methylene blue be dropped.

(8) That the subject of "Tuinol" be continued.

(9) That the subject of antihistamines be reassigned.

(10) That the Associate Referee's recommendation that collaborative study be undertaken of the application of the infrared technic outlined in his report to the determination of nitroglycerin, meperidine, codeine, and amidone be approved. It is also recommended that the subject of spectrophotometric methods be divided between two Associate Referees, one on infrared methods and the other on spectrophotometry at other wavelengths. It is further recommended that the Associate Referee on the latter subject submit to collaborative study the method for the determination of isonicotinyl hydrazide, *This Journal*, **36**, 722 (1953).

MISCELLANEOUS DRUGS

It is recommended—

(1) That the tables for identification of various drugs by their microscopic crystallographic properties given in the Associate Referee's reports be adopted, first action, and that the subject be continued.

(2) That the following topics be continued:

Mercury compounds
Organic iodides and separation of halogens
Alkali metals

COSMETICS

It is recommended—

(1) That the following methods, now first action, be adopted as official:

Urea in deodorants
Chlorides in deodorants
Sulfates in deodorants
Methenamine
Phenolsulfonates
Thioglycollate solutions
Qualitative tests for potassium bromate and sodium perborate.

(2) That the following topics be continued:

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

COAL-TAR COLORS

It is recommended—

(1) That the chemical and spectrophotometric methods for the determination of 4-toluene-azo-2-naphthol in D&C Red No. 35 outlined in the Associate Referee's report be submitted to collaborative study.

(2) That the method for the determination of higher sulfonated dye in FD&C Yellow No. 6 outlined in the Associate Referee's report be adopted, first action.

(3) That methods for the determination of subsidiary dyes in FD&C Red No. 2 outlined in the Associate Referee's report be submitted to collaborative study.

(4) That further collaborative study of paper chromatography of coal-tar colors be undertaken.

(5) That the present first action methods for the determination of intermediates derived from phthalic acid, lower sulfonated dyes in FD&C Yellow No. 5, and Lake Red C Amine in FD&C Red Nos. 8 and 9, be adopted as official.

(6) That the present method for determination of the boiling range of amines (pseudocumidine) derived from FD&C Red No. 1 be deleted, and that the topic be discontinued.

(7) That all methods for ether extracts in coal-tar colors presently designated as first action (**34.52,** and **34.54–34.58,** inclusive) be made official, and that the topic of ether extracts in coal-tar colors be discontinued.

(8) That the subject of halogens in halogenated fluoresceins be discontinued.

(9) That the following topics be continued:

Heavy metals in coal-tar colors
Sulfonated amine intermediates in coal-tar colors
Volatile amine intermediates in coal-tar colors
Non-volatile unsulfonated amine intermediates in coal-tar colors
Inorganic salts in coal-tar colors
Sulfonated phenolic intermediates in coal-tar colors
Unsulfonated phenolic intermediates in coal-tar colors
Intermediates in triphenylmethane colors
Arsenic and antimony in coal-tar colors
Lakes and pigments.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

A. H. ROBERTSON (State Food Laboratory, Department of Agriculture and Markets, Albany, N. Y.), *Chairman;* SAMUEL ALFEND, and FRANK A. VORHES, JR.

GENERAL RECOMMENDATION

The Committee recommends that in view of the forthcoming revision of *Methods of Analysis*, each General Referee should review his chapter thoroughly for editorial and other corrections and should study all methods now in first action status with a view to resolving their status, either by adoption as official or by deletion.

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 study of the rapid method described in the Associate Referee's report, for determination of residual catalase activity in frozen vegetables, be subjected to collaborative study.

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

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

(5) That work on methods for determination of moisture in dried vegetables be continued.

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

COFFEE AND TEA

It is recommended—

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

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

DAIRY PRODUCTS

It is recommended—

(1) That work on an alternative procedure for the preparation of samples of frozen desserts which contain insoluble material, such as fruits, nuts, etc., be discontinued.

(2) That the methods described by the Associate Referee for the determination of acidity and sucrose in ice cream and frozen desserts be subjected to collaborative study.

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

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

(5) That studies be made on substitutes for sulfuric acid in the Babcock method for fat in milk and cream, to include comparison with the results by the Roese-Gottlieb method; and that additional work on modification of the Babcock method proposed by the Associate Referee be postponed until the method substituting a detergent for sulfuric acid can be evaluated.

(6) That work on the detection and estimation of foreign fats in dairy products be continued and expanded by the appointment of additional Associate Referees to pursue the work along several lines, including study of the physical details of the Reichert-Meissl method.

(7) That the method for critical temperature of dissolution for distinguishing oleomargarine from butter, *This Journal*, **33**, 495 (1950), be adopted, first action.

(8) That studies on the detection of reconstituted milk be made.

(9) That the procedure for sampling cheese, **15.123**, be restudied as suggested by the Referee.

(10) That studies on the freezing point of milk be conducted on authentic herd milk, and on such milk after pasteurization and homogenization.

(11) That the study of the procedure for preparation of samples of pressurized cream for analysis be continued, and include study of the use of a surface cooler accessory, and collaborative work.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That the first action method, *This Journal*, **36**, 77 (1953), for glycerol in eggs be made official.

(2) That work on the determination of ammonia nitrogen in eggs be continued along the lines indicated by the Associate Referee, *This Journal*, **34**, 346 (1951).

(3) That the official method for cholesterol, **16.13–16.16**, be amended as recommended in the Referee's report and adopted as official.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES)

It is recommended—

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

(2) That studies of succinic, lactic, and volatile acids as indices of decomposition in tomatoes caused by molds be discontinued; but that studies of these acids as indices of bacterial decomposition in tomatoes be continued.

(3) That study of the relationship of pigment changes to decomposition in strawberries be discontinued.

(4) That search be continued for indices of decomposition in fruits and vegetables.

(5) That study of biological and chemical methods for histamine in fish as an index of decomposition be continued.

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

(7) That study be initiated on the correlation of 3,4-dihydroxyphenylacetic acid with insect filth in cereal foods.

(8) That the rapid method for determination of water-insoluble fatty acids in cream and butter, *This Journal*, **36**, 1077 (1953), be adopted, first action.

(9) That studies of uric acid as an index of filth in nuts, cereals, and eggs be continued.

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

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

GELATIN DESSERT PREPARATIONS AND MIXES

It is recommended—

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

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the method for the determination of total solids in fish described in the Associate Referee's report be modified by inserting before the last sentence the following: "or preheated forced draft oven for one hour at 100°C., set for full draft," and be adopted, first action.

(2) That the method for determining total solids in oysters in a forced

draft oven, as described by the Referee, be adopted, first action, after eliminating the sentence "Make duplicate determinations."

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

(4) That the forced draft air oven method as adopted for oysters be studied for other molluscs, such as scallops, clams, and mussels, and for crustaceans, such as lobsters and crabs.

(5) That the forced draft air oven method as adopted for fish be studied for crustaceans such as lobsters and crabs.

(6) That collaborative work on the rapid method for ether extract in canned fish described by the Associate Referee be extended to fish other than salmon.

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 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.

(3) That the extent of interference by other insecticides such as Dilan, Rhothane, and Dimite, in the colorimetric determination of DDT be investigated, and that work on the Stiff-Castillo method be discontinued.

(4) That the qualitative method for 1080, as revised in the Referee's report, be adopted, first action.

(5) That the first action quantitative method for 1080 as revised in the Referee's report be adopted as official.

(6) That the method for methoxychlor described in the Associate Referee's report be subjected to collaborative study.

(7) That the collaborative study of the Schechter-Hornstein method for benzene hexachloride be continued; and that work on the beta-isomer be dropped.

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

(9) That the typographical error in the method for fluorine be corrected by substituting "0.75 ml" for "0.5 ml," **24.48,** example at the end of first paragraph, page 396.

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

(11) That chemical and enzymic methods for the determination of trace amounts of phosphorus-containing insecticides, including parathion, be studied.

(12) That study of chemical and flame photometric methods for the determination of sodium in foods be continued.

GUMS IN FOODS

It is recommended—

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

(2) That studies on the detection of alginates and other gums in cheese and cheese spreads be continued.

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

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

(5) That work be continued on the detection of alginates and other stabilizing agents in dressings for food.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That work be continued on the determination of starch in meat products.

(2) That the serological method for detection of horsemeat be studied collaboratively.

(3) That studies of chemical methods for detection of horsemeat, including spectrophotometric procedure, be continued.

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

(5) That the method for crude fat in meats using petroleum ether, *This Journal*, **36**, 288 (1953), be studied collaboratively.

(6) That methods for determination of lactose in meat products in the presence of maltose be studied.

NUTS AND NUT PRODUCTS

It is recommended—

(1) That the methods for moisture, crude fat, crude protein, crude fiber, and ash be subjected to further collaborative study.

(2) That methods for sucrose, reducing sugar, and sodium chloride be further studied.

(3) That methods for the detection and determination of hydrogenated oil in nut products be studied.

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

(5) That the first action method for added starch in peanut butter be further studied.

MICROBIOLOGICAL METHODS

The Referee has recommended reconsideration of the Association's policy with respect to development and adoption of microbiological methods, because of (1) the concurrent development and publication of

such methods by other organizations such as the American Public Health Association, (2) the qualitative nature of most of the methods, (3) the difficulty in applying customary exact collaborative procedures, and (4) the fact that most of these methods are useful chiefly to industry, and are not forensically useful in regulatory work.

When this Association initiated work on microbiological methods, there was a real need for efforts to establish procedures definitely agreed upon, which were lacking at that time. Since then, this Association, the American Public Health Association, and the National Canners Association have developed recognized procedures, and the field of usefulness of these methods has been well delineated.

It is the view of this Committee that this Association should not work on microbiological methods which are not useful in regulatory work, and which are sponsored by other organizations, but only on methods for which there is definite need in regulatory work.

It is therefore recommended—

(1) That the functions of the Committee to Confer with the American Public Health Association on Standard Methods of Milk Analysis be broadened to include consideration of any other methods of concern to both Associations, and that its title be changed to "Committee to Confer with the American Public Health Association on Methods of Analysis."

(2) That this Committee confer with the American Public Health Association as to microbiological methods on which this Association will continue work, and on those which the American Public Health Association will publish.

(3) That the first action methods for sugars and canned vegetable products should be dropped.

(4) That the first action methods for eggs and egg products be studied collaboratively.

MICROCHEMICAL METHODS

It is recommended—

(1) That studies on catalytic combustion methods for bromine and chlorine be discontinued for the present.

(2) That the first action catalytic combustion method for sulfur, with titrimetric determination of sulfates, be subjected to further collaborative study.

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

(4) That studies on the Dumas method for nitrogen, including work on rapid methods as suggested by the Referee, be continued.

(5) That work on a micro-Kjeldahl method for determination of nitrogen in materials containing N-N and N-O groups be discontinued until

studies on the Dumas method show whether there is substantial need for such a method.

(6) That additional collaborative studies be made on methods for determining acetyl groups.

(7) That additional studies on alkoxyl methods, as recommended by the Referee, be made.

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 fats and oils be continued.

(3) That further work be done on methods for antioxidants in fats and oils.

SPICES AND OTHER CONDIMENTS

It is recommended—

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

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

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

(4) That studies on the determination of sorbitol in vinegar be continued.

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

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

(7) That the procedure for preparation of samples of french dressing, *This Journal*, **35**, 86 (1952), be amended by inserting after the first paragraph the directions for preparation of samples in large containers, given in the Associate Referee's report.

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

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

ENZYMES

It is recommended—

(1) That the enzymatic method for organic phosphate and carbamate insecticides, described by the Referee, be studied collaboratively, and that an attempt be made to make the method specific for some insecticides.

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

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

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

FLOYD ROBERTS (State Laboratories Department, Lock Box 900, Bismarck, North Dakota), *Chairman;* KENNETH L. MILSTEAD, and ROBERT A. OSBORN

MALT BEVERAGES, BREWING MATERIALS, AND ALLIED PRODUCTS

It is recommended—

(1) That methods for the determination of sugars in beer be studied.

(2) That the study of the wet-ash orthophenanthroline method for iron in beer be continued.

(3) That the first action direct non-ash procedure for iron in beer, adopted two years ago, be modified to provide for the use of α,α'-dipyridyl as an optional reagent and that the method be further studied.

(4) That the direct non-ash method of Stone for copper in beer be studied collaboratively.

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

(6) That work on methods for the measurement of turbidity in beer be resumed.

(7) That the method for alpha-amylase in malt, as presented in the report of the Associate Referee, be adopted, first action.

WINES

It is recommended—

(1) That collaborative studies of the applications of paper chromatography in wine analysis be discontinued.

(2) That the spectrophotometric method for the determination of phosphates in wines and spirits, *This Journal,* **35,** 257 (1952), be made official.

(3) That the spectrophotometric method for the determination of tannins in wines and spirits, *This Journal,* **35,** 255 (1952), be adopted, first action, to replace **9.38** which was deleted, first action.

(4) That studies on the determination of color in wines be discontinued.

(5) That the study of methods for the determination of tartrates in wines be continued.

(6) That the method for "total alkalinity of the ash" be studied collaboratively.

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

DISTILLED LIQUORS

It is recommended—

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

(2) That the study of the methods for methanol in distilled liquors and drugs be continued with a view of correlating the procedures.

(3) That the investigation of higher alcohols in distilled spirits by chromatography be discontinued.

(4) That a study be made to adopt the "Dye Color Method for Beer," **10.3,** for use as a color reference solution for distilled liquors.

(5) That a study of the naturally occurring sugars in aged liquors be undertaken.

CORDIALS AND LIQUEURS

It is recommended—

(1) That the method for citric acid in fruits and fruit products, *This Journal,* **34,** 75 (1951), be adopted, first action.

(2) That the method for tartaric acid in fruit and fruit products, *This Journal,* **36,** 79 (1953), be adopted, first action.

(3) That the method for total malic acid in fruit and fruit products, *This Journal,* **36,** 80 (1953), be further studied.

CACAO PRODUCTS

It is recommended—

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

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

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

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

CEREAL FOODS

It is recommended—

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

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

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

(4) That the methods on soybean flour for moisture, **13.63,** ash,

13.64, nitrogen, **13.65,** with substitution of "15" for "10" so as to read "using 15 g K_2SO_4 or Na_2SO_4," crude fiber, **13.66,** and oil or petroleum ether extract, **13.67–13.68,** be made official.

(5) That the method for determination of egg content in bakery products by the sterol-digitonin method referred to in the report of the Referee be adopted, first action, and the study continued.

(6) That the sterol-digitonin method for determination of the sterol content of noodles as described in the report of the Referee this year be adopted, first action and the study continued.

(7) That the Armstrong method for the determination of bromate in flour referred to in the report of the Associate Referee be studied collaboratively.

(8) That the study of the procedure for lipoids and method I for lipoid P_2O_5, *This Journal,* **36,** 76 (1953), be continued.

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

(10) That the study of methods for choline in noodles be discontinued.

(11) That the study on moisture determinations be continued.

BAKING POWDER

It is recommended—

(1) That the work as reported in *This Journal,* **34,** 60, 296 (1951) on the neutralizing value of monocalcium phosphate be continued.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

It is recommended—

(1) That method **35.9** for the determination of sediment in milk be changed editorially and a description of the filtering apparatus included as proposed by the Associate Referee, and that the method so changed be adopted, first action.

(2) That the title of the method, *This Journal,* **35,** 96 (1952), for "Identification of Tomato Rot Fragments" be changed to "Rot Fragments in Comminuted Tomato Products."

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

(4) That work be continued on extraneous material in peanut butter.

(5) That work be continued on extraneous materials in spices.

(6) That the method **(35.87)** for extraneous materials in ground capsicums, as amended, *This Journal,* **36,** 90, 301 (1953), be further amended as proposed by the Associate Referee and adopted, first action.

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

(8) That a study of methods for the determination of extraneous materials in eggs be undertaken.

(9) That the study of methods for the determination of internal insect infestation of wheat be continued.

(10) That the revised pancreatin digestion method, *This Journal*, **36**, 309 (1953), for insect fragments and rodent hairs be studied collaboratively.

(11) That methods for identification of insect contaminants in food and drug products be continued.

(12) That method **35.28 (a)** be changed as proposed by the Associate Referee and that the revised method be adopted, first action.

(13) That the revised method for "Direct trapping to show insect fragments and rodent hairs contributed by wheat or mill" proposed by the Associate Referee be studied collaboratively.

(14) That the title of section **35.32** and **35.33** be changed to read "Whole and Degerminated Corn Meal, Corn Grits, Rye Meal, Wheat Meal, Whole Wheat Flour, Farina, and Semolina."

(15) That the revised method proposed by the Associate Referee "Insects, Insect Parts, and Rodent Hairs" be adopted, first action, and that methods **35.29 (b)**, **35.33**, **35.34**, **35.35**, and **35.36 (b)**, **(c)**, and **(d)** be dropped.

(16) That section **35.36 (a)** be changed editorially as proposed by the Associate Referee and be adopted, first action.

(17) That the method for the determination of heavy and light filth in granulated nut meats be adopted, first action and that method **35.22 (c)** be dropped.

(18) That the method for "Light Filth in Spanish Peanuts," *This Journal*, 35, 94 (1952), be designated as "Light Filth in Peanuts with Adhering Testa."

(19) That the first procedure in section **35.22 (b)** for light filth in shelled nuts beginning "Weigh 100 g into suitable beaker" and ending "Trap off, filter, and examine microscopically" be deleted. This method essentially duplicates the method which follows it and is less satisfactory from the standpoint of filth recovery.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That official methods **19.4** and **19.5** for vanillin and coumarin be deleted, first action.

(2) That the official Winton method for Lead Number, **19.8**, be dropped, first action.

(3) That the procedure Color Value, **19.17**, be dropped, first action.

(4) That the procedure Residual Color after Precipitation with Lead Acetate, **19.18**, be dropped, first action.

(5) That ultraviolet methods for vanillin and coumarin be further studied.

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the method for total malic acid, laevo and inactive, presented in the report of the Associate Referee be adopted, first action.

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

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

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

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That the qualitative test for P-4000 as reported by the Associate Referee be adopted, first action.

(2) That the quantitative method for P-4000 as reported by the Associate Referee be further studied.

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

(4) That further work on the determination of monochloracetic acid in fruit juices and beverage bases be discontinued.

(5) That the method for "Determination of Quaternary Ammonium Compounds as Reineckates," *This Journal*, **35**, 456 (1952), be adopted, first action.

(6) That the method for quaternary ammonium compounds in milk as reported by the Associate Referee be adopted, first action.

(7) That the method for quaternary ammonium compounds in water solutions as reported by the Associate Referee be adopted, first action.

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

(9) That the qualitative method for benzoates and hydroxybenzoates as reported by the Associate Referee this year be submitted to collaborative study.

(10) That the qualitative test for fluorides as reported by the Associate Referee be adopted, first action.

(11) That the method for the determination of dimethyldichlorosuccinate, *This Journal*, **36**, 539 (1953), be studied collaboratively.

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

(a) Methods for the determination of dulcin.
(b) Methods for the determination of hydrogen peroxide in dairy products.
(c) Methods for the determination of diphenyl in fruit peel and juice.

SUGARS AND SUGAR PRODUCTS

It is recommended—

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

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

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

(4) That the method for carbohydrate analysis of honey using carbon column pretreatment referred to in the report of the Associate Referee this year be submitted to collaborative study.

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

(6) That the study of methods **29.132–29.154,** inclusive (starch conversion products), be continued.

(7) That the method described in the report of the Associate Referee this year for the determination of the color of raw cane sugars be adopted, first action.

(8) That the collaborative study of refractive indices of sucrose solutions in the range 60 to 70 per cent be continued.

(9) That the work on Somogyi phosphate method be discontinued.

(10) That the anthrone method referred to in the Associate Referee's report for the determination of very small amounts of glucose be further studied.

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

(12) That collaborative work be carried out on the determination of sucrose by determining reducing sugars by the Lane and Eynon method before and after hydrolysis.

(13) That work be continued on development of methods for the detection of adulteration of maple products and the identity of maple concentrates.

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-SEVENTH ANNUAL MEETING, OCTOBER 12, 13, AND 14, 1953

The changes recorded in the methods of the Association, as given below, become effective, as provided in Section 8 of the by-laws, on the thirtieth day from the date of publication of this Report, February 15, 1954.

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

1. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

2. FERTILIZERS

(1) The following method for water-soluble magnesium in coarse particles was adopted as first action:

Weigh 15 g sample of unground fertilizer using spoon to convey the material. Transfer to 250 ml beaker, add 100 ml H_2O, cover with watch glass and boil for 30 min. Disintegrate lumps by rubbing with rubber bulb of medicine dropper. Pour thru sieve with openings of 420 microns, washing beaker and sieve with stream of tap H_2O thru rubber tubing attached to faucet. A 3 in. sieve is most convenient. Transfer residue on sieve to porcelain evaporating dish, disintegrate lumps with the rubber bulb, and again wash on sieve. Repeat process until sepn is complete; 3 repetitions are usually sufficient. Do not force particles thru by rubbing on the screen. Wash final residue into 250 ml volumetric flask with H_2O, let stand until clear and decant as much H_2O as possible, retaining all mineral particles in flask. Det. Mg as in **2.55** or **2.56** beginning with, "Add 30 ml of HNO_3 and 10 ml of HCl, and boil 30 min." in **2.55**, and report as % Mg in original sample.

3. SOILS

No additions, deletions, or other changes.

4. CAUSTIC POISONS

No additions, deletions, or other changes.

5. ECONOMIC POISONS

(1) The title of this chapter was changed to "Pesticides."

(2) The following first action methods were adopted as official:

(a) Total arsenic III., **5.8–5.9** (p. 52).
(b) Distillation method for fluorine, **5.21–5.22** (p. 57).
(c) Fluorine present as sodium fluosilicate, **5.23–5.24** (p. 58).
(d) Total arsenic oxide, **5.37–5.38** (p. 60).
(e) Sulfide sulfur, indirect method, **5.124** (p. 76).

(3) The following statement was added to method **5.143** (p. 81), Chloramine T, after "Cl." in the last line: "To convert active Cl to available Cl, multiply active Cl by 2."

(4) The first action method for Pyrethrin II, **5.112** (p. 73), as revised, *This Journal*, **35**, 64 (1952), was adopted as official.

(5) The first action method for Pyrethrin I in Pyrethrum Extracts in Mineral Oil, as it appears in **5.113–5.114** (p. 73) was adopted as official.

(6) The first action method for the determination of total chlorine in esters of 2,4-D and 2,4,5-T in liquid herbicides by the Parr bomb-boric acid procedure, *This Journal*, **36**, 67 (1953), was adopted as official.

(7) The first action method for the fungicidal test, **5.166–5.170** (p. 91), was revised as follows and adopted as first action:

(a) In the second line of **5.167** change "*p*H 5.6–5.8" to "*p*H 6.1–6.3."

(b) Section **5.169** was revised to read as follows:

5.169 PREPARATION OF CONIDIAL SUSPENSION

Prep. petri dish agar cultures by planting inoculum at center of agar plate and by incubating culture at 25–30° for 10, but not more than 15 days. Remove mycelial mats from surface of 5 agar plate cultures by means of sterile spatula or heavy flattened wire. Transfer to a heat sterilized glass tissue grinder (A. H. Thomas Company, size B) and macerate with 25 ml sterile physiological NaCl soln (0.85% NaCl) or to a heat sterilized Erlenmeyer flask contg 25 ml sterile saline with glass beads and shake thoroly. Filter suspension thru sterile absorbent cotton to remove hyphal elements. Estimate density of conidial suspension by counting in a haemacytometer and store as stock spore suspension (125–155 million conidia/ml), at 2°C. to 10°C. for periods up to 4 weeks and use in prepg test suspensions of conidia. Standardize test conidial suspensions as needed by diluting stock spore suspension with physiological NaCl soln so that it contains 5 million conidia/ml.

6. PLANTS

(1) The following ion exchange resin method for the preparation of solutions for sugar determinations, *This Journal*, **36**, 402 (1953), was adopted as first action:

REAGENTS

(a) *Cation exchange resin.*—Amberlite IR-120 (H) analytical grade.

(b) *Anion resin.*—Duolite A-4.

(c) *Celite analytical filter aid.*

(d) *Dextrose.*—U. S. Bureau of Standards.

DETERMINATION

Place aliquot of the alc. ext., **6.48(a)** in beaker and evap. on steam bath to drive off alcohol. Avoid evapn to dryness by adding H_2O. When odor of alcohol has disappeared, add ca 15–25 ml of H_2O and heat to 80°C. to soften gummy ppts and break up insol. masses. Cool to room temp. Prepare a thin mat of Celite on filter paper in Büchner funnel or on sintered glass filter and wash until H_2O comes thru clear. Filter sample thru Celite mat, wash mat with distd H_2O and make filtrate and washings to an appropriate vol. in a volumetric flask. Mix well and label soln A.

Place 50.0 ml aliquot of soln A in 250 ml Erlenmeyer flask, add 2 g of IR-120 cation and 3 g of A-4 anion ion-exchange resins. Allow to stand 2 hrs with occasional swirling. Take a 5 ml aliquot of the de-ionized soln and det. sugar as directed under **29.61**, **29.62**, and **29.63**.

(2) The following method for the spectrochemical analysis of plant material was adopted as first action:

SPECTROCHEMICAL ANALYSIS OF PLANT MATERIAL

The methods described cover determination of the major elements and the common minor elements in plant material where spectrochemical methods are valuable because of the considerable number of elements which may be determined simultaneously. However, the basic instrumental techniques described are not limited to analysis of this material. With appropriate modifications of sample preparation and details of standards composition both of the methods may be adapted to many analytical problems involving other agricultural or biological materials.

The descriptions illustrate the wide latitude permissible between methods capable of satisfactory performance. Two entirely different instrumental and photometric techniques are described. Points in both methods may be helpful and suggestive in instances where the available equipment does not permit complete adherence to the details of either. The section on general considerations is intended to be supplementarily helpful in such cases. Method 1 involves direct comparison of the emission values for samples and standards of very similar composition. The spectra are prepared by d.c. arc excitation under chemically buffered conditions. Method 2 employs the internal standard technique, a.c. spark excitation, and a correction system for matrix differences and thus permits more leeway between sample and standard similarity. This represents an advantage in analysis of batches of samples which might vary considerably in individual composition.

Substitutions in apparatus for either method may be made if based upon approximately equivalent performance.

METHOD 1. D.C. ARC EXCITATION

Apparatus.—Motor generator power source; Bausch and Lomb large Littrow prism spectrograph, equipped with custom built device for magnetically rotating the arc envelope; Eastman Kodak plates III-0 or K33, and facilities for their controlled processing; lathe for machining electrodes; Applied Research Laboratories densitometer.

Electrodes.—Use standard grade spectrographic carbon rods, $\frac{5}{16}$" diam. Cut the rods into segments $\frac{1}{4}$" long and drill a tapered hole in one end, for mounting on a pedestal for burning. In the other end drill a crater 7 mm in diam. and 4 mm deep; machine wall thickness to 0.4 mm. Purify the electrodes by preburning with ca 2.5 mg of buffer until the buffer is consumed. Use high purity carbon rods, $\frac{1}{4}$" diam., for the upper electrodes.

Buffer.—Mix equal parts, by weight, of purified Li_2SO_4 and pure spectrographic graphite powder.

Preparation of standards.—Make an exploratory analysis of the particular batch of samples to be analyzed. Prepare a stock mixture from individual pure chloride solns of the respective elements, adjusting concns in the mixture so that they will be equivalent to 0.1 of the highest concn of each element in the samples. Adjust acidity to 1 ml HCl per 100 ml. Make successive dilns, each 40 or 50% of the previous strength, to produce a series of element concns covering the indicated sample ranges. Use HCl (1–100) in preparing the dilns. (It is important that the major element levels be in the same general proportions to each other in these standards as they are in the samples.)

Transfer accurately measured 0.1 ml portions of the standards to electrode craters which have been waterproofed as subsequently directed. Place the electrodes containing the aliquots in an oven and dry at 100°C.

Treatment of samples.—Grind the plant material in a Wiley mill fitted with a 40 mesh sieve. Accurately weigh 10 mg portions of oven dried sample and transfer to the electrode craters. Set the charged electrodes into holes drilled in a transite (asbestos cement) block. Ash the material by placing the block in a muffle furnace and bringing the temp. slowly up to 500°C.

Waterproof the cooled electrodes containing the ash by dropping around each crater edge 4 drops of a lukewarm soln containing 12% paraffin-kerosene and allow to air dry. Add 1 drop distd H_2O and 2 drops of HCl (1–30) to each crater to convert salts to chlorides. Dry the treated electrodes at 100°C.

Excitation.—Add 4 mg of buffer to each of the electrodes containing the salt residues from samples and standards. Align electrodes in holders and burn to completion with a current of 150 volts and 24 amperes. During burning period magnetically rotate the gaseous envelope of the arc to increase homogeneity, and maintain electrode alignment by periodic adjustment. Place a stepped sector in light beam to permit simultaneous determination of the nine elements.

Photometry.—Process the photographic plates under uniformly controlled conditions. Use the following analysis lines: Ca, 2997.3; Mg, 2779.8; P, 2553.3; Mn, 2801.1 or 3054.4; Fe, 3020.6; Al, 3082.2; Na, 3302.3; Cu, 3247.5 or 3274.0; and B, 2496.8 or 2497.7 Angstroms.

Obtain line transmittances for each element with densitometer. Construct analysis curves for each plate by plotting transmittance values for standards against their known concns. Evaluate sample spectra in relation to standards on the same plate. Replicate the series of exposures 3 or 4 times, each replication being recorded on a different plate.

METHOD 2. A.C. SPARK EXCITATION

Apparatus.—Applied Research Laboratories spark unit; Applied Research Laboratories 1.5 meter grating spectrograph with enclosed spark stand; Eastman Kodak Spec. Anal. #1 film; Applied Research Laboratories film processing equipment; electrode drill; Applied Research Laboratories densitometer; and Applied Research Laboratories calculating board.

Electrodes.—Purify standard grade spectrographic carbon rods, ¼″ diam., by successive digestions with HCl (1+1), HNO_3 (1+1), and distd H_2O. Dry in an oven. Cut the purified rods into 2″ lengths. Drill a crater 5 mm in diam. and 6 mm deep in one end and pack crater with a portion of the carbon removed in drilling. Opposing electrodes consist of the purified rods pointed in a pencil sharpener. A flat tip $\frac{1}{16}$″ in diam. is produced by a pin stop in the sharpener.

Hydrochloric acid-cobalt dissolving soln.—Dil. 20 ml 2% Co soln and 300 ml HCl to 2 liters.

Standardization and reference procedure.—(The procedure outlined under "Preparation of Standards" below is designed to cover analysis of miscellaneous plant materials of various compositions. The reference standards used are, therefore, of a general nature. If requirements are confined to analysis of a specific and reasonably uniform type of plant material the standardization procedure may be simplified to satisfy only the variations involved. In such cases the proportional composition and concentrations suggested for the standards should be adjusted to match approximately the material to be analyzed. In practice the spectra of 10 samples and duplicates of 2 reference standards are recorded on a film. The series of exposures is duplicated on another film. Averages of ratio values from the two films are used in all cases.)

Preparation of standards.—Prepare stock solns of each element from pure chemicals. The following concentrations are convenient: K, 5.0 and 0.5%; Ca, 3.0 and 0.3%; Mg and P, 1.0 and 0.1%; Mn, Fe, Al, Zn, and Na, 0.5, 0.05, and 0.005%; Cu and B, 0.05, 0.005, and 0.0005% (5 ppm); and Co, 2.0%.

Make a stock mixture, for use in preparing general standards, by combining the following amounts of the element solns and diluting to 500 ml:

K	50 ml of 5.0% = 0.5%	Al	5 ml of 0.5% = 50 p.p.m.
Ca	50 ml of 3.0% = 0.3%	Zn	5 ml of 0.5% = 50 p.p.m.
Mg	35 ml of 1.0% = 0.07%	Mn	2 ml of 0.5% = 20 p.p.m.
P	35 ml of 1.0% = 0.07%	Cu	5 ml of 0.05% = 5 p.p.m.
Fe	5 ml of 0.5% = 50 p.p.m.	B	5 ml of 0.05% = 5 p.p.m.

The relation between standards and samples is based upon the chloride residue from 1 g of sample dissolved in 5 ml of the HCl-Co dissolving soln. Therefore, for each ml of stock mixt. evapd and so dissolved the resulting standard corresponds to the percentages of the various elements that are listed above.

Prepare a series of standards by evaporating 1, 2, 4, 7, and 10 ml aliquots of the stock mixture with 2 ml HCl and dissolve each residue in 5 ml of HCl-Co soln. Use curves prepd from these general standards for all of the elements except Ca and P. (Ratios for these two elements are affected by the progressive increase of matrix elements in the series.)

Prep. standards for the P curve as follows: Make 5 mixtures from the element stock solns, each containing sample equivalents of 2.5% K, 1.5% Ca, and 0.3% Mg. Add 1, 2, 4, 6, and 8 ml of 0.1% P soln, respectively. Add 2 ml HCl to each, evap., and dissolve as above. These standards represent 0.1%, 0.2%, etc. of P in the constant matrix.

Prep. standards for Ca curves at different K levels as follows: To 5 mixtures, each contg sample equivalents of 1.0% K, 0.3% Mg, and 0.4% P, add 1, 2, 4, 8, and 12 ml of 0.3% Ca soln, respectively. Evap. and dissolve as previously directed. These standards represent 0.3%, 0.6%, etc., of Ca, and are used to obtain a Ca curve for a K level of 1.0%. Prep. three more sets of Ca standards by the same procedure, raising the K levels in the respective sets to 2.0%, 3.5%, and 5.0%.

As different K and Ca levels affect the fiducial point rather than the slope of the P curve, the 0.4% P present in all of the Ca standards serves to indicate the relative fiducial adjustment necessary for the various amounts of these elements present.

Prep. low, medium, and high standards, used routinely as references for the analysis scales, from 2.5, 5, and 10 ml portions of the stock mixt. (Addnl standards of any desired composition, for checking curve relationships and matching unusual samples, may be easily prepd from the element solns.)

Treatment of samples.—Weigh an amount of the dry, ground material that will produce an ash weighing preferably between 0.06 and 0.10 g. One g of av. plant material meets this requirement. Ash at 550°C. Digest ash with an excess dil. HCl and evap. to dryness on a hot plate. Treat residue with 5 ml of HCl-Co soln and warm until salts are dissolved. Allow any insoluble material to settle out, or remove it by passing the soln thru a small, dry filter.

Preparation of electrodes.—Add, drop by drop, ca 0.1 ml of sample or standard soln to a packed electrode and allow to soak in. Dry treated electrodes at 130°C. for 2 hrs and keep in desiccator until sparked.

(The rapidity with which the soln soaks into the electrode influences the general density of the spectrum. Individual electrodes, although prepared in as nearly the same way as possible, may vary considerably in this respect. Where absorption is very rapid, add an addnl drop of soln, so that some will remain on top of the electrode for several min. before being absorbed. If surface of powdered carbon is glazed in the packing operation no soln will penetrate, and the electrode should be discarded.)

(While a spectral density level equivalent to 25–30% transmittance for the Co reference line is desirable from the standpoint of uniform element range coverage,

a density range between 20 and 50% transmittance for this line does not affect the validity of the element to Co ratios. For this reason density differences encountered between duplicate exposures provide an excellent addnl check on emulsion calibration.)

Excitation.—Align and space electrodes 1/8" apart in the holders with a jig, and spark for 25 sec. (This period is usually sufficient to empty the crater; disregard any remaining material.) Maintain source input at 235 volts and the primary at 90 volts. The power setting on the unit is 2 KVA and the secondary inductance position 4, resulting in estimated capacitance of .021 microfarad and inductance of 360 microhenries. Use a slit width of 40 microns and an aperture which will permit 14

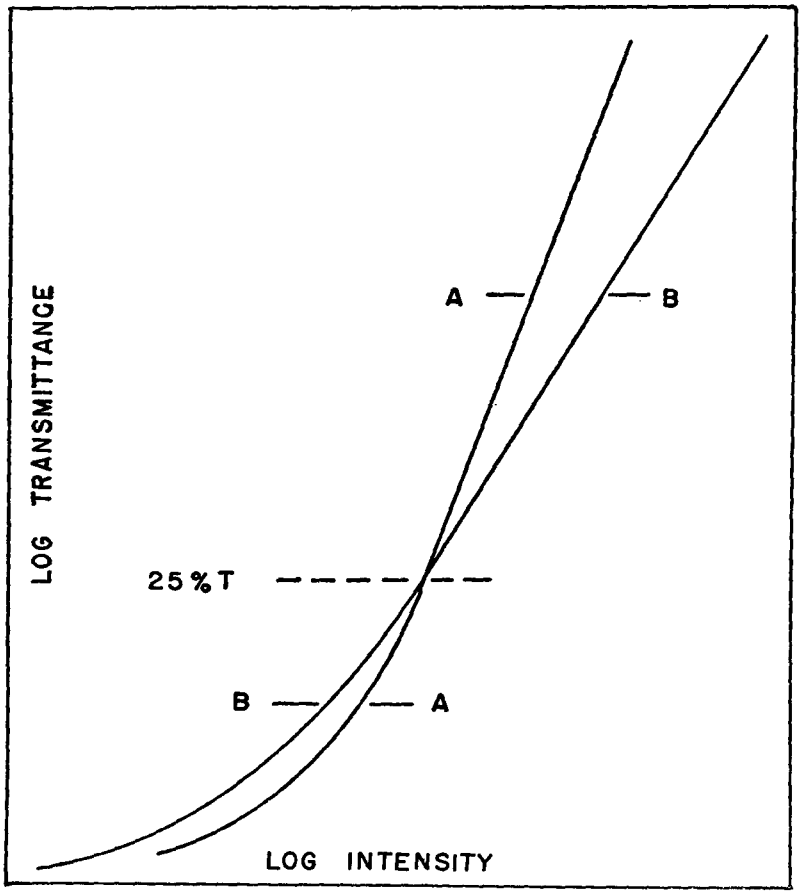

FIG. 1.—Positioning of correlated emulsion curves. A—region around 4050A, used for potassium T settings. B—region around 3000A, used for 3044 reference and all other analysis lines. The characteristics and relation of the curves shown are illustrative.

exposures and a clear strip for densitometer settings on the film. Adjust gates so that total background, in general, is slight. Use no lens or filter. Make no background correction.

Emulsion calibration.—Prep. electrodes under sample conditions, substituting 2% soln of Fe in dil. HCl for sample soln. Place a stepped filter or sector in light beam and spark for 10 sec.

Record transmittances of the related steps of several lines of various densities, in the vicinity of 3000A. Plot readings against known relative intensities, using calculating board. Integrate and smooth curve sections to form regular emulsion curve. Construct a second curve, to be used for lines 4044 and 4047 only, in the same manner, from stepped intensities of lines in that portion of the spectrum. Position the 2 curves on calculating board to cross at 25% on the logarithmic transmittance scale. (Fig. 1.)

In practice check curves by comparison of lines in the plant standards' spectra, the relative intensities of which have been established in direct relation to the above calibration. (A further check is afforded by density differences between duplicate exposures, as previously mentioned.)

Film processing.—Develop film for 3 min. at 70°F. in D-19 developer, wash, and fix with Kodak X-ray fixer. These operations are done in rocking tanks, thermostatically controlled. Spray wash, sponge, and dry film in an infrared dryer.

Photometry.—Use following analysis lines: K, 4044 or 4047; Ca, 2997; Mg, 2781 or 3337; P, 2553 or 2555; Mn, 2949, 2933, or 3460; Fe, 3020.6 or 3021.1; Al, 3082; Zn, 3345; Na, 3302; Cu, 3274; B, 2498; and Co, (reference line) 3044 Angstroms.

Set densitometer scale at 100% transmittance on the clear film at each pertinent wavelength, and obtain the analysis line transmittances. Using calculating board, determine log I element/Co ratios, for all of the elements except K, from the regular emulsion curve. Obtain K/Co ratios by positioning the Co line transmittance on the regular emulsion curve and the transmittance for line 4044 or 4047 on the correlated emulsion curve for that region.

Plot respective ratios against the known percentages of the elements in the standards and draw analysis curves. Ratios from the sample spectra may be referred directly to these curves, if desired, to obtain analysis values. Due to the considerable number of curves involved in analysis of miscellaneous plant materials, however, it is more convenient to arrange the data obtained from these curves as percentage scales for each element.

Mount a blank cardboard strip parallel to the horizontal logarithmic scale on the calculating board, which is allowed to represent log I ratios. Mark the complete range of percentage points on the strip in alignment with the corresponding ratios on the logarithmic scale. The fiducial relationship is adjustable by sliding the strip in its mounting.

On the strip for Ca place the several scales that are relative to different levels of K. On the P strip place a series of replicas of the P scale in fiducial alignment relative to different levels of combined K and Ca.

Correlate all of the analysis strips with the low, medium, and high general standards and mark percentage levels of these standards on the strips as fiducial reference points. Make fiducial adjustment for each set of duplicate films by aligning these reference points with the respective average ratios for the standards thereon. Refer accompanying samples to these settings.

In practice, determine K first. With the K percentage known, the proper scale to be used for Ca is indicated. With both K and Ca contents known, the correct scale for P is indicated. Make no correction for matrix variation for the other elements.

3. GENERAL CONSIDERATIONS

Instrumental technic.—If, because of equipment limitations, neither of the above methods described can be followed in detail, or if the particular analytical problem involves detn of other elements, the following procedure is recommended: Determine experimentally, to the limits of the facilities available, the potential of various electrode prepns and excitation conditions with relation to element detectability and general concn requirements. If a set of conditions shows promise, make a preliminary check for repeatability of line indices.

For detn of the very minute amounts of some of the elements in plant material, preliminary chemical sepn and concn may be necessary. (A satisfactory procedure using 8-hydroxyquinoline is described by Mitchell.*) The trace element concentrate so obtained may be combined with a suitable matrix and the subsequent treatment adapted to the regular instrumental technic.

Selection of analysis lines on the basis of desirable intensity and freedom from spectral interference by other elements is facilitated by preparing a spectrum of each component element at the av. concn level at which it occurs in the material to be analyzed. Align the spectra collectively for comparison, preferably by exposure on the same film or plate.

Precision.—Standardize all conditions of the technique and det. the reproducibility of results by running a suggested 20 successive exposures on a sample of representative composition. For each element calc. the standard deviation of a single exposure and divide by the square root of the number of individual exposures that will be averaged in practice to constitute a detn. From this estimate of the standard deviation of a single detn, calc. the coefficient of variation for each element. The following upper limits for precision error of spectrographic detns in analysis of plant material are tentatively suggested as being satisfactory in relation to other routine methods, or to practical requirements: Coefficients of variation for K, Ca, and P, 5.0; Mg, Mn, Fe, Al, Na and Cu, 10.0; B, 15.0. Methods 1 and 2 are potentially capable of meeting these requirements within their scopes.

Accuracy.—A precise technic is essential, but it is by no means the only factor involved in ultimate accuracy. The reliability and appropriateness of the standards and the judgment used in the reference procedure are of utmost importance. Failure in any of these respects can result in serious calibration error for an otherwise satisfactory method.

Synthetic standards should be carefully prepared from top grade moisture-free analyzed chemicals, collectively blanked for minor and trace elements. Values assigned natural standards should preferably be confirmed by more than one laboratory.

The necessity for matrix similarity between standards and samples, or for a closely controlled correction system for matrix differences, has been stressed in the methods and is again emphasized. Correction scales should be frequently checked against standards which closely match the particular types of plant materials being analyzed.

It should be remembered that the precision error of the technique applies to reference exposures as well as to the samples. For this reason, fiducial adjustments should be based upon as many reference exposures as may feasibly be included in each run of samples.

7. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

* MITCHELL, R. L., "The Spectrographic Analysis of Soils, Plants and Related Materials," Commonwealth Bureau of Soil Science, Harpenden, England, 1948.

8. BEVERAGES: NON-ALCOHOLIC AND CONCENTRATES

No additions, deletions, or other changes.

9. BEVERAGES: DISTILLED LIQUORS

(1) The procedure "Characteristic acids—preparation of sample," **9.56** (p. 136), and the first action method for citric acid, **9.58** (p. 137), (as revised, *This Journal*, **34**, 61 (1951), were revised as follows and the revised method for citric acid was adopted as first action:

(a) In method **9.56** delete the last three words "as directed below." and substitute the first eleven lines of the section headed "Determination," *This Journal*, **36**, 79 (1953), when citric and tartaric acids are to be determined.

(b) For the present sentence under **9.58** substitute "Proceed as in *This Journal*, **34**, 75 (1951), under 'Citric acid (normal).' "

(2) The first action method for tartaric acid, **9.57**, was revised by substituting for the present sentence the directions "proceed as in *This Journal*, **36**, 79 (1953) under 'Determination' line 12, 'Transfer 100 ml clear filtrate. . . . ' "

(3) The first action method for phosphates in wines and spirits, *This Journal*, **36**, 70 (1953), was adopted as official.

(4) The following method for the determination of tannin in spirits and wines was adopted as first action to replace the present first action method, **9.38**, which was deleted, first action:

REAGENTS

Same as **9.37**.

DETERMINATION

Pipet 1 ml of wine or whisky into 100 ml volumetric flask contg 75 ml of H_2O. Add 5 ml of Folin-Denis reagent, 10 ml Na_2CO_3 soln, and dil. to 100 ml with H_2O. Shake well, and det. absorbance after 30 min. at 760 mμ. If the absorbance for a 1 cm cell is greater than 0.9, make a 5:1 diln of sample before taking a 1 ml aliquot, and proceed as above.

Calculations.—Absorbance = mg tannic acid/100 ml (from curve).

$$\text{Grams tannic acid/100 ml spirits} = \frac{\text{mg tannic acid/100 ml}}{1000} \times \text{reciprocal of dilutions}$$

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

(1) The following method for alpha-amylase in malt was adopted as first action:

REAGENTS

(a) *Special starch.*—Use Merck's soluble Lintner starch, special for diastatic power determination.

(b) *Beta-amylase.*—The special beta-amylase powder free from alpha-amylase made by the Wallerstein Laboratories, 180 Madison Avenue, New York, N. Y.

should be used. This prepn has been standardized to 2000°L. and should comply with following specifications. At addn level used, there must not be a variation greater than 5% in dextrinization of standard malt infusion when 1 and 3-day old substrates are compared. Further, a substrate prepd by adding twice the level of beta-amylase indicated must not deviate by more than 5% from that prepd with the recommended level after 24 hrs standing. Store powder in tightly closed bottle in the refrigerator. To avoid moisture condensation on the cold enzyme prepn, allow bottle to warm to room temp. before opening.

(c) *Stock iodine soln.*—Dissolve 5.50 g I crystals (A.C.S.) and 11.0 g KI in H_2O and dil. to 250 ml. Store in dark bottle and make fresh soln monthly.

(d) *Dil. iodine soln.*—Dissolve 20.0 g KI in H_2O, add 2.00 ml of stock I soln and dil. to 500 ml. A series of 13×100 mm test tubes contg 5 ml of the dil. I soln must be made up beforehand and attemperated at 20° in readiness for testing. An all glass automatic pipet such as the Machlett type is recommended for rapidly dispensing this soln.

(e) *Buffer soln.*—Dissolve 120 ml glacial acetic acid and 164 g anhyd. Na acetate in H_2O and dil. to 1 l.

(f) *Sodium chloride soln.*—0.5%. Dissolve 5 g reagent NaCl in a l of H_2O. This soln need not be made up in a volumetric flask.

(g) *Buffered limit-dextrin (alpha-amylodextrin) substrate.*—Prep. a suspension of 10.00 g (dry weight) of Merck's soluble starch in cold H_2O and pour slowly into boiling H_2O. Boil with stirring for 1 or 2 min., cool, add 25 ml of buffer soln, and 250 mg of beta-amylase dissolved in a small amount of H_2O. Make up to 500 ml, sat. with toluene, and store at or close to 20°C. for not less than 18 hrs nor more than 72 hrs before use.

APPARATUS

(a) *Constant temperature bath.*—Set at 20 ±0.05°C.

(b) *Reference color standard.*—Use the special Alpha-Amylase Color Disc (catalog 620-S5) made by Hellige Inc., 3718 Northern Blvd., Long Island City, New York.

(c) *Comparator.*—Either the standard Hellige comparator (catalog 607) or the pocket comparator (catalog 605) with prism attachment (catalog 605-A) may be used. The comparator shall be illuminated with a 100 watt frosted lamp mounted in such a manner that direct rays from the lamp do not shine in the operator's eyes. The lamp shall be placed 6 inches from the rear opal glass of the comparator.

(d) *Comparison tubes.*—Use precision bore square tubes with 13 mm viewing depth. A tube filled with distd H_2O should be placed behind the color disc.

The alpha-amylase color disc is correct only when used with the specified 13 mm viewing depth. Precision bore square tubes are specified to obviate need for individual calibration of test tubes and to insure use of standard viewing depth. The 13 mm precision square tubes are supplied as standard equipment with the Hellige Comparator and are also used with the Coleman Universal Spectrophotometer. They may be secured from either Hellige, Inc., distributors of the Coleman instrument, or Fisher and Porter Co., Hatboro, Penn.

DETERMINATION

Preparation of malt infusion.—Ext. 25 g (±0.05 g) of finely ground malt exactly as in **10.62**, paragraph 1, but use 500 ml of 0.5% NaCl soln instead of distd H_2O. Dil. 20 ml of malt infusion to 100 ml with 0.5% NaCl soln.

Dextrinization.—The buffered limit-dextrin substrate and dild malt infusion should be attemperated and dild to final vol. at 20°C.

Transfer 20.0 ml of substrate soln at 20°C. to a 50 ml Erlenmeyer flask, add 5 ml of 0.5% NaCl soln and again adjust temp. to 20°C. Add 5 ml dild malt infusion at 20°C., blowing it in and counting time from the instant the first of the dild malt infusion reaches the starch substrate in flask. After 10 min. reaction time, add 1 ml of hydrolyzing mixt. to 5 ml of dil. I soln at 20°C., shake, pour into the 13 mm square tube and compare with the alpha-amylase color disc in the comparator.* At appropriate intervals remove addnl 1 ml aliquots of the hydrolyzing mixt., add to dil. I soln, mix, and compare with color disc until the alpha-amylase color is reached.†

During the initial stages of the reaction it is not necessary that the 1 ml sample be measured precisely before addn to the dil. I soln. As the end-point is approached make the addn accurately with a 1 ml pipet. (A fast flowing pipet such as a 1 ml bacteriological pipet is recommended for withdrawing the 1 ml aliquot.) Blow contents of the pipet into the I soln. Near the end-point, take readings every 0.5 min. on the min. or half min. In case two readings 0.5 min. apart show that one is darker than the alpha-amylase color disc and the other is lighter, then the end-point is recorded at the nearest quarter min. Shake out the 13 mm square tube used for color comparison between successive readings.

For accuracy and convenience it is desirable that dextrinization times fall between 10 and 30 min. With malts of low alpha-amylase activity it may be necessary to use 10 ml of the dild infusion. In this case, do not add 5 ml of the NaCl soln. The final vol. of the reaction mixt. should always be 30 ml.

CALCULATION OF ALPHA-AMYLASE ACTIVITY

From the time interval necessary for dextrinization, and the wt of malt represented by the infusion aliquot taken, calc. alpha-amylase units. An alpha-amylase unit is defined as the quantity of alpha-amylase which will dextrinize soluble starch in the presence of an excess of beta-amylase at the rate of one g per hr at 20°C.

$$20°\ D.U.\ (\text{as is basis}) = \frac{24}{W \times T}$$

$$20°\ D.U.\ (\text{dry basis}) = \frac{D.U.\ (\text{as is}) \times 100}{100 - M}$$

Where: W = wt in g of malt in aliquot taken; T = dextrinizing time in min.; M = % moisture in sample; and 24 is the wt of starch employed (0.4 g) multiplied by 1 hr (60 min.).

Example:

W = 0.05 g; T = 20 min.; Temp. = 20°C.

$$20°\ D.U.\ (\text{as is}) = \frac{24}{0.05 \times 20} = 24.0$$

Report dextrinizing units to nearest 0.1 unit.

(2) The first action direct, non-ashing method for iron in beer, *This Journal*, **35**, 68 (1952), was revised as follows and adopted as first action:

* Slight differences in color discrimination between different operators may be minimized by the use of the prism attachment, maintenance of a 6–10 inch reading distance between the eye and the comparator, and by the experience gained with continued practice.

† Care should be taken to prevent the tubes containing the reaction mixture plus I from changing temp. while comparing colors. If color comparisons are made immediately following the addn of reaction mixt. to I, there will be essentially no temp. change and no change in color.

Change Reagent **(3)** to:

(3) *Color reagent.*—Either 0.2% α,α'-dipyridyl **(a)** or 0.3% *o*-phenanthroline. **(b)** may be used.

(a) Dissolve 1 g α,α'-dipyridyl (2,2′-bipyridine) in 20 ml of acetic acid (1+2), dil. to 500 ml with H_2O.

(b) Dissolve 1.5 g *o*-phenanthroline H_2O in 500 ml of H_2O heated to not over 80°C.

Under "Procedure," line 4, change "*o*-phenanthroline" to "color."

11. BEVERAGES: WINES

(1) The first action method for the determination of phosphates in wines and spirits given above, Chapter 9, item (3), was adopted as official.

(2) The method for the determination of tannins in spirits and wines given above, Chapter 9, item (4), was adopted as first action.

12. CACAO BEAN AND ITS PRODUCTS

No additions, deletions, or other changes.

13. CEREAL FOODS

(1) The following methods for soybean flour (p. 208) were adopted as official:

(a) Moisture, **13.63.**

(b) Ash, **13.64.**

(c) Nitrogen, **13.65,** with the substitution of "15" for "10" so as to read " . . . 15 g of K_2SO_4 or . . . ."

(d) Crude fiber, **13.66.**

(e) Oil or petroleum ether extract, **13.67–13.68.**

(2) Methods **13.35,** Lipoids, and **13.36,** Lipoid phosphoric acid (P_2O_5), were deleted, final action.

(3) The following digitonin method for the determination of the sterol content of noodles was adopted as first action:

Weigh 5 g of sample (ground to pass at least 20 mesh sieve) into 300 ml Erlenmeyer flask and add, with shaking, 15 ml of HCl (1+1) in such a manner as to keep the particles on the sides at a min. Heat on steam bath 30 min., shaking flask frequently to break up any lumps and insure hydrolysis of all the sample. While cooling inclined flask under tap, add carefully, with swirling, 15 g KOH pellets at such a rate that liquid may boil, but not so violently as to cause loss by spurting. Cool, add 20 ml of 95% alcohol by rinsing down sides of flask, and heat on steam bath for 45 min. with air condenser, shaking frequently. Add 25 ml of H_2O by rinsing down sides, mix well, and cool. Add 50 ml ether, swirl mixture vigorously 1 min. and transfer to 500 ml separator. Wash flask with 25 ml and 10 ml portions of ether and with 50 ml of 1% KOH soln, pouring washings into separator in a slow stream while gently swirling liquid and continue gentle swirling 10–15 sec. Allow liquid to sep. and slowly draw off soap soln into the 250 ml separator, but do not draw off any small quantity of emulsion or of insoluble matter at interface. Rinse down sides of 500 ml separator with 5 ml of 1% KOH soln and draw this off into smaller separator. Add 25 ml ether to smaller separator and shake vigorously ca 1 min. After liquids have sepd discard lower layer. Add ether layer to soln in larger separator, rinsing the 250 ml separator with 10 ml of ether. Wash ether soln as before with 3 more suc-

cessive 50 ml portions of 1% KOH, still retaining any insoluble matter or emulsion in separator. Wash ether soln twice by swirling with 50 ml of H_2O. Finally draw off as much of H_2O as possible without loss of ether soln. Add a porcelain chip to 300 ml Erlenmeyer flask, transfer ether to flask, rinse separator successively with three 5 ml portions of ether, and rinse stem of separator with ether. Add rinsings to flask and evap. ether on the steam bath.

Dissolve residue in 5 ml of acetone, filter with suction, if necessary, into 100 ml centrifuge tube or test tube under a bell jar, thru Knorr type extn tube contg a medium porosity fritted glass, covered with a few g of washed and ignited sand. Wash flask and tube 3 times with 4 ml portions of acetone and rinse tube and stem with a few ml of acetone (vol. not over ca 20 ml). Add 5 ml of freshly prepd digitonin in 80% alcohol contg 40 mg digitonin (hasten soln of the digitonin by warming to ca 40–50°C. under a hot H_2O tap). (Products contg more than 6% egg yolk solids or equivalent (moisture free basis) require addnl digitonin soln or use of an aliquot portion for the pptn.) Mix by rotation. Place a porcelain chip in the tube, suspend tube in a steam bath with a small amount of steam to avoid boiling or spattering over, evap. nearly to dryness, add 50 ml hot H_2O (near boiling), and stir well with glass rod to disperse ppt and dissolve the excess digitonin. Place tube in a boiling H_2O bath and hold several min. with frequent stirring, cool to ca 60°C., add 25 ml of acetone, mix well by stirring, cool to room temp. in a beaker of cold tap H_2O. When the ppt has nearly all settled (ca 15 min.) remove glass rod, rinsing off any adhering ppt with acetone. Decant into previously dried and weighed Gooch crucible (preferably 10 ml capacity) contg an asbestos pad covered with ca 1 g of washed and ignited sand. Using wash bottle, wash tube several times with a few ml portions of acetone to transfer all the ppt (*Caution:* avoid transfer of any particles of chips). Finally rinse crucible with acetone to dissolve any fat-like material, rinse with 5 ml of ether, dry for 0.5 hr at 100°C. and weigh. (Check wt after a second 0.5 hr of drying.) The wt of residue times 0.243 equals the wt of sterol. Report as % sterol on a moisture free basis.

(4) The following digitonin method for the determination of the sterol content of bakery products was adopted as first action:

See method for the determination of sterol content of noodles, item (3) above, using 5 g air dried sample.

14. COFFEE AND TEA

No additions, deletions, or other changes.

15. DAIRY PRODUCTS

(1) The following method for the determination of the critical temperature of dissolution for the differentiation of oleomargarine from butter (*This Journal*, **33**, 495 (1950)) was adopted as first action:

REAGENTS

(a) *Alcohol reagent.*—Mix 2 vol. 95% (by vol.) ethyl alcohol (checked by sp.gr.) with 1 vol. redistd (b.p. 128°–132°) iso-amyl alcohol, both measured with volumetric pipet or flask. Keep well stoppered.

APPARATUS

(a) *Test tubes.*—Pyrex, 18×150 mm, calibrated by adding H_2O from a buret at marks of 2 ml and 4 ml.

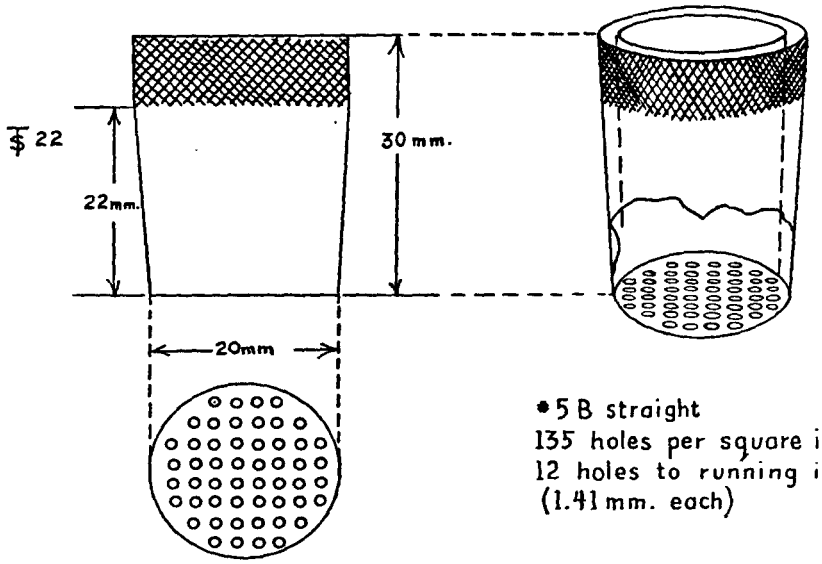

FIG. 1

FIG. 2

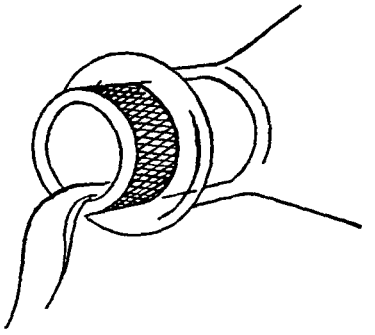

FIG. 3

FIGS. 1, 2, and 3.—Filter sieve.

(b) *Micro burner.*

(c) *Pipet.*—Glass tube of ca 2–3 ml capacity, drawn to a fast-flowing tip.

(d) *Thermometer.*—Range 0°–100°C., graduated in degrees.

DETERMINATION

Prep. oil from butter or oleomargarine as in 15.115. Oil must be clear. Fill test tube to 2 ml mark with oil, using pipet. Immediately add alcohol reagent to the 4 ml mark (or add 2 ml with pipet). Using thermometer as stirring rod, mix the two layers and heat in flame of micro burner. Continue stirring and heating until the mixt. becomes clear and homogeneous. *Do not boil.* Remove from heat, continue

stirring until a definite turbidity appears in the *mixture proper*. Record temp. at first discernible turbidity. (Opalescence will immediately follow thruout entire mixt. with a further drop in temp.)

(2) The following rapid method for the determination of water-insoluble fatty acids in cream and butter, *This Journal*, **36**, 1077 (1953), was adopted as first action:

REAGENTS

(a) *Sodium ethylate.—See* **16.29(b).** (95% alcohol or methyl alcohol may be substituted for absolute alcohol and K may be substituted for Na.)

PREPARATION OF SAMPLE

(a) *Cream.*—Weigh 20 g into 125 ml glass-stoppered Erlenmeyer flask, standard taper No. 22. Add 25 ml ice-cold H_2O, cool to 10°C. and shake until butterfat separates in granular form. Discard if the granular fat conglomerates into one lump.

(b) *Butter.*—Weigh 10 g into 125 ml glass-stoppered Erlenmeyer flask, standard taper No. 22, warm to melt butter and cool until butter is in a thick creamy consistency. Add 50 ml ice-cold H_2O, shake, cool to 10°C. and shake for ca 5 sec.

DETERMINATION

Insert filter sieve (see Figures) and pour off serum layer. Add 50 ml ice-cold H_2O, insert glass stopper and shake for ca 5 sec. Pour off liquid thru inserted filter sieve. Wash 3 addnl times. Dissolve the washed butter in 25 ml of ethyl ether, pour into small separatory funnel, wash the Erlenmeyer with few ml of ether and add to separatory funnel. Allow to settle for a few min. and drain off the H_2O-curd layer. Draw ether-fat soln into 125 ml Erlenmeyer flask, wash separatory funnel with a few ml of ether, add to Erlenmeyer and titrate with 0.05 *N* Na ethylate, **(a)** using phenolphthalein as indicator. Compute WIA in mg per 100 g of fat.

1 ml of 0.05 *N* Na ethylate = 13.5 mg of WIA.

16. EGGS AND EGG PRODUCTS

(1) The official method for cholesterol, **16.13–16.18** (p. 277), was revised as follows and the revised method adopted as official:

(a) **16.13(c)**: change to read: "*Ether.*—U.S.P. or A.C.S., free of peroxides."

(b) **16.13(d)**: change to read: "*Dried ether.*—Immediately before use shake peroxide-free ether with anhyd. $CaCl_2$ equal to 10% of the vol. of the ether and filter."

(c) **16.15**: delete "Approx." from line 7 from bottom of page 278.

(d) **16.16(b)**: delete "Add 4–5 g of Br" and substitute "Add 0.6 ml Br from a graduated 1.0 ml pipet."

(e) **16.16(f)**: Add at end of paragraph: "Reagent or commercial NaOCl soln, 5%, checked for concn as above, is also satisfactory."

(2) The first action method for the determination of glycerol in eggs, **16.27–16.28** (p. 283), as revised, *This Journal*, **36**, 77 (1953), was adopted as official.

17. ENZYMES

No additions, deletions, or other changes.

18. FISH AND OTHER MARINE PRODUCTS

(1) The following method for the determination of total solids in fish was adopted as first action:

Prep. a 9 cm flat-bottom covered weighing dish in the following manner: cut into short lengths and add ca 2 g asbestos fibers of the type used in prepg Gooch crucibles and an 8 cm stirring rod with flattened end. Dry dish, asbestos, and rod in air oven at 100°C. 1 hr, cool, and weigh. Weigh into dish to nearest mg, 9–10 g of prepd sample. Add 20 distd H_2O and mix sample intimately with asbestos. Support end of stirring rod on edge of dish and evap. just to dryness on steam bath, stirring once while still moist. Drop rod into dish and heat 4 hrs in an oven at 100°C. or in preheated forced draft oven set for full draft for 1 hr at 100°C. Cover dish, cool in desiccator, and weigh promptly.

(2) The following alternate method for the determination of total solids in oysters using the forced draft oven was adopted as first action:

Weigh quickly 10 g of meats, liquid, or mixed meat and liquid, in flat-bottom metal dish ca 9 cm in diam. Spread sample evenly over bottom of dish, insert directly in preheated forced draft oven set at full draft and dry 1.5 hrs at 100°C. Cool in desiccator and weigh promptly.

19. FLAVORING EXTRACTS

(1) The following methods were deleted, first action:

(a) Official gravimetric method for vanillin and coumarin, **19.4–19.5** (p. 305).
(b) Official Winton method for lead number, **19.8** (p. 306).
(c) The procedure for color value, **19.17** (p. 307).
(d) The procedure for residual color after precipitation with lead acetate, **19.18** (p. 307).

20. FRUITS AND FRUIT PRODUCTS

(1) The following method for total malic acid, laevo and inactive, was adopted as first action:

(Either or both iso-citric acid and tartaric acid may be present.)

REAGENTS

(a) *Thymol blue indicator.*—**34.79(k).**
(b) *Phenolphthalein.*—1% in alcohol.
(c) *Solvent.*—Either 30% tertiary amyl alcohol, or 30% *n*-butyl alcohol in chloroform. (Eastman's Practical tert-amyl alcohol and U.S.P. $CHCl_3$ without further treatment have been found satisfactory.)
(d) *Silicic acid suitable for chromatography.*—Analytical reagent 100 mesh (No. 2847 Mallinckrodt Chemical Co. or equivalent).

APPARATUS

(a) *Chromatographic tubes.*—Ca 13 mm internal diam. and 400 mm long; may have a perforated disc or coarse sintered disc at beginning of constriction. Glass piston to fit tube.
(b) *Source of pressure*—**18.11(c).**

STANDARDIZATION OF SILICIC ACID COLUMN

Mix in mortar to uniform powder 6 g of silicic acid and the amount of 0.5 *N* H_2SO_4 that will allow solvent to be eluted at rate of 1–1.5 ml per min. with a pressure of less than 1 atmosphere. The amount of 0.5 *N* H_2SO_4 required may vary with different batches of silicic acid; however, the silicic acid and 0.5 *N* H_2SO_4 must be measured accurately and the column for the detn made up exactly as under standardization. (3 ml of 0.5 *N* H_2SO_4 has been found satisfactory for one batch of silicic acid.) Slurry with enough $CHCl_3$ to fill chromatograph tube. Place a small amount of cotton at bottom of tube and pour slurry into tube so that no air bubbles are occluded. Cut disc of filter paper with a cork borer to fit tightly inside tube and with piston, pack the silicic acid until no more $CHCl_3$ is forced out. A column packed in this manner permits the sample to be stirred with solvent without disturbing column and also gives a sharper sepn of acids. Remove piston, pour the remaining $CHCl_3$ out top of the tube and place 10 ml cylinder under the tube. Dissolve ca 10 mg malic acid in 1 ml 5 *N* H_2SO_4 in small beaker. Stir with 2 g of silicic acid—or enough to make a free flowing powder that does not adhere to beaker. Transfer thru funnel to column, rinse beaker with ca 5 ml of solvent (c), and pour thru funnel into tube. With long, thin rod stir powder and solvent in tube until all air bubbles are removed. Remove rod and stand it in sample beaker. With pressure, pack sample until solvent just disappears into jel. Rinse the long rod, beaker and funnel with ca 2 ml solvent and sink into jel. Repeat washing with another 2 ml of solvent. Place plug of cotton in top of tube, wet it with solvent and with rod push it down to top of sample. Fill tube with solvent and apply pressure so that eluate is forced out at rate of 1–1.5 ml per minute. Titrate eluate in 10 ml portions, rinsing cylinder with 10 ml CO_2-free H_2O and using thymol blue indicator. If the mixt. being titrated is swirled gently so that no emulsion is formed the end point is sharp and easily seen. When excess acid is present the indicator goes into the lower layer and as neutrality is reached, the indicator turns yellow and enters the aq. phase. Swirl and add alkali until lower layer is colorless and aq. layer is blue. Note amount of solvent (c) required to bring the malic acid to bottom of jel and the amount required to elute all the malic acid. In the same manner, det. the threshold vol. of citric acid. The vol. of solvent necessary to bring citric acid to bottom of column should be at least 20 ml more than that required to elute all the malic acid. During elution, the column will become semi-transparent at the top and progressively downward, but when malic acid is all eluted, 1 cm or more of the column should be unchanged in appearance. When the semi-transparency reaches the bottom of the column, H_2SO_4 may be carried into the eluate. (It has been found, using the above procedure, that the first 70 ml of eluate contained no malic acid and that the next 70 ml contained all the malic acid and no citric acid. However, the vol. required should be detd for the app. and the particular batch of silicic acid used.)

DETERMINATION

Proceed as in tartaric acid method, *This Journal*, **36**, 79 (1953) to line 12 under "Determination." (The 30% KOH and device for filtering at 0° are not used.) Conc. 200 ml of filtrate—do *not* neutralize—to ca 15 ml. Transfer with a small amount of H_2O to a tared beaker with bottom ca 3 cm diam. and evap. on steam bath to 1 g (±.05 g). A jet of air over surface of liquid may be used to hasten the evapn but no portion of the bottom of the beaker should be allowed to dry, as darkening of soln may occur with loss of malic acid. Cool, add 0.25 ml H_2SO_4 (1+1), 2 g silicic acid or slightly more if necessary to make a free flowing powder, and transfer to a prepd column as under standardization. Discard the vol. of eluate equal to the found threshold vol. of malic acid and collect in a 150 ml beaker the eluate that will

contain all the malic acid and none of the citric. Evap. solvent on steam bath. (A jet of air over liquid hastens evapn and prevents loss by bumping.) Dissolve residue in ca 10 ml CO_2-free H_2O and titrate with 0.02 *N* NaOH using phenolphthalein indicator. Correct titration for a blank on the eluate put thru a column as under detn. 1 ml 0.02 *N* NaOH = 1.34 mg malic acid; mg malic acid ÷ 0.64 = total malic acid in sample.

Acidify soln contg the neutralized malic acid with a drop of *N* acetic acid, evap. to ca 15 ml, transfer to a 25 ml volumetric flask and proceed as under "Laevo-malic Acid" beginning at line 5. *This Journal*, **36**, 80 (1953). Total malic acid in sample minus laevo-malic acid = inactive malic acid.

21. GELATIN, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or other changes.

22. GRAINS AND STOCK FEEDS

(1) The method for crude fat in baked dog food adopted first action last year, *This Journal*, **36**, 80 (1953) was replaced by the following method which was adopted as first action:

Place 2 g of ground, well mixed sample in Mojonnier fat extn tube, add 2 ml of alcohol to prevent lumping on addn of acid. Shake so as to moisten all particles. Add 10 ml of HCl (25 + 11), mix well, set tube in H_2O bath held at 70–80°, and shake at frequent intervals 30–40 min. Fill to within 1–2 ml of mark with alcohol and cool. (Level of liquids should be in neck of Mojonnier tube just below pouring-off level.) Add 25 ml of ether, stopper flask with glass, cork, Neoprene or a good quality rubber stopper thoroly cleaned with alcohol, and shake vigorously for 1 min. Release pressure carefully after tube is shaken so that none of the solvent, containing fat, is lost. Wash adhering solvent and fat from stopper back into extn tube with few ml of petr. ether. Add 25 ml of redistd petr. ether (b.p. below 60°) and again shake vigorously for 1 min. Let stand until upper liquid is practically clear, or centrifuge Mojonnier tube 20 min. at ca 600 r.p.m. Pour off as much as possible of the ether-fat soln thru filter consisting of pledget of cotton packed just firmly enough in stem to allow free passage of ether into 150 ml beaker contg several glass beads. Rinse lip of Mojonnier tube with a few ml of petr. ether. Re-ext. liquid remaining in tube twice, each time with only 15 ml of each ether. Shake 1 min. on addn of each ether. Draw off clear ether soln thru filter into same beaker as before and wash tip of Mojonnier tube, stopper, funnel, and end of funnel stem with few ml of mixt. of the 2 ethers in equal vol. Evap. ethers slowly on steam bath, then redissolve the dried fat residue in four 10 ml portions of ethyl ether, filtering each portion thru small fat-free filter paper into weighed 100 ml beaker, contg several glass beads, that has been previously dried at 100°, cooled in desiccator, and then weighed. Use a fifth 10 ml portion of ether for rinsing filter paper and funnel. Evap. ethers on steam bath, dry 90 min., cool in desiccator and weigh as soon as room temp. is reached. Make blank detn on reagents.

(2) The official method for crude protein in feeds, **22.10** (p. 343) was revised to read as follows:

Det. N as directed under **2.24** using Hg as the preferable catalyst in case of material digestible with difficulty. Multiply results by 6.25, or in the case of wheat grains by 5.70.

(3) The following method for the determination of total solids in highly acid milk byproducts was adopted as first action:

Add ca 2 g of ZnO, A.C.S. (freshly ignited or oven dried) to flat-bottom dish not less than 5 cm in diam. Weigh. Add ca 1 g sample and weigh quickly. Add ca 5 ml distd H_2O and distribute sample evenly on bottom of dish. Heat on steam bath, exposing max. surface of dish bottom to live steam, until apparently dry. Heat in air oven at 98–100° 3 hrs or until constant in wt. Cool in desiccator, then weigh quickly. Calc. wt of residue. Titrate acidity of sample and calc. as lactic acid **(15.14)**. To compensate for moisture formed when acid is neutralized by ZnO, add 0.1 g to residue wt for each g of acid (as lactic) in the weighed sample. Report % residue (corrected) as total solids.

23. MEAT AND MEAT PRODUCTS

No additions, deletions, or other changes.

24. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

(1) The first action quantitative method for 1080, **24.74–24.80** (p. 413), was revised as follows and adopted as official:

In section **24.77(b)**, p. 414, line 4, after the word "convenient)." insert, "Or' more quickly, centrifuge and decant the supernatant liquid." Delete the last part of this paragraph beginning with line 6, "If only small continuous . . . hastens evapn.)"

(2) The following qualitative test for 1080 (*This Journal*, **34**, 828 (1951) was adopted as first action:

REAGENTS

(a) *Decolorizing* carbon.—*See* **15.9(b)**.

(b) *Thiosalicylic acid soln.*—Dissolve 300 mg thiosalicylic acid (Eastman's tech. grade is suitable) in a mixt. of 2 ml *N* NaOH and 18 ml H_2O.

(c) *Potassium ferricyanide soln.*—Dissolve 1 g $K_3Fe(CN)_6$ in H_2O and make up to 50 ml with H_2O.

DETERMINATION

Follow directions in **24.77–24.78** for sample prepn and ether extn. If convenient, ext. sample of sufficient size to obtain 2–10 mg of 1080. With very low levels of 1080, *e.g.*, 1–5 p.p.m., ext. sample of sufficient size to obtain at least 0.5 mg of 1080. Sep. ether ext. from any aq. sludge which may have been carried over in the extn, add ca 5 g anhyd. Na_2SO_4 and 0.5 g decolorizing carbon per 100 ml ether, and shake vigorously. Allow to stand ca 15 min. at room temp. with occasional shaking, and decant thru fluted filter into separatory funnel. Add ca 25 ml H_2O and sufficient NaOH soln (ca *N*) to make aq. layer alk. after vigorous shaking (outside test paper). Draw off aq. layer into 125 ml Erlenmeyer flask and aerate to remove dissolved ether. Using *p*H test paper and ca *N* solns of H_2SO_4 and NaOH, adjust to *p*H 4–6. Add 0.5 g carbon and place on steam bath for 15 min. Cool under tap and filter thru fluted filter into test tube ca 25 mm×150 mm in size. Add 1 ml of thiosalicylic acid soln, 2 drops 1+1 NaOH, and mix. Conc. soln to small vol. by placing on steam bath under a gentle current of air. Completely dry residue in drying oven at 130°C. or, if time is not a factor, in a 100°C. oven. (When convenient, overnight drying is satisfactory, with or without prior concn of the soln.) Dissove the *thoroly* dry residue in 2–3 ml H_2O, add 1 ml of potassium ferricyanide soln, and mix. The appearance of a red color is a positive test for 1080. A red ppt forms at once when 1 mg or more of 1080 is present, or upon standing when only a fraction of a mg is present.

Employ chromatographic instead of carbon purification in the following instances:

1. With pineapple juice when less than 2 mg of 1080 can be extd.
2. With grape juice even when 2 mg or more of 1080 can be conveniently extd.
3. With any food or material when 1080 is strongly suspected and a negative test is obtained using the carbon purification technic.

For chromatographic purification, follow **24.79** for separating 1080 from other acids. Discard forerun which may contain acetic acid and other extraneous materials. Collect percolate fraction large enough to contain all the 1080 as detd by a preliminary run. Ext. fluoroacetic acid from chromatographic percolate with 25 ml H_2O and sufficient alkali to cause aq. layer to retain alkalinity after vigorous shaking (outside test paper). Draw off organic layer and discard. Draw off layer into 125 ml Erlenmeyer flask and aerate to remove $CHCl_3$. Pour soln into test tube and continue as in procedure with carbon purification, beginning with "Add 1 ml of thiosalicylic acid soln . . . ."

(3) The official method for fluorine was corrected by changing in section **24.48** (p. 396), line 5, "0.5 ml" to "0.75 ml."

25. NUT AND NUT PRODUCTS

No additions, deletions, or other changes.

26. OILS, FATS, AND WAXES

No additions, deletions, or other changes.

27. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The following qualitative test for propoxy-2-amino, 4-nitrobenzene (P-4000) was adopted as first action:

To 100 ml sample, add 10% NaOH dropwise, until just alk. (*p*H 7.5–8.0), and ext. 3 times with 25 ml portions of petr. ether, shaking 2 min. each time. Wash combined ether extracts with 5 ml H_2O, discard H_2O, and transfer ether ext. to small beaker or dish, and allow petr. ether to evap. spontaneously to ca 5 ml. Transfer remainder to 25 ml test tube, wash beaker with several small portions of petr. ether, and transfer washings to test tube. Evap. carefully to dryness. Add 2 ml HCl (1+1), then add 2 ml satd $SnCl_2$ soln. Immerse test tube in boiling H_2O bath for 15 min., remove and cool. Add dropwise strong Br-H_2O to excess. If P-4000 is present, the soln will change from nearly colorless to a purplish-red or burgundy red color, and if a considerable excess of Br-H_2O is added, the soln will turn yellow. (NOTE: If the soln is not nearly colorless when removed from H_2O bath, it should be dild until nearly colorless before the Br-H_2O is added.)

(2) The following qualitative test for fluorides was adopted as first action:

REAGENTS

(a) *Aluminum soln.*—Dissolve 2.22 g $AlNH_4(SO_4)_2 \cdot 12H_2O$. in H_2O, add 3 drops HCl, and make up to 250 ml with H_2O.

(b) *Oxine reagent.*—Dissolve sufficient 8-hydroxyquinoline in 2 *N* acetic acid to make a 5% soln. 1 ml of this soln is equivalent to ca 5 ml of the Al soln.

(c) *Ammonium acetate soln.*—Dissolve 77 g of NH_4 acetate in H_2O and make up to 500 ml with H_2O.

(d) *Aluminum 8-hydroxyquinolate.*—Warm 250 ml of Al soln to 50–60° and add excess of oxine reagent. Add NH_4 acetate soln slowly until a permanent ppt forms. Then add 20–25 ml more to insure complete pptn. Allow ppt to settle and filter thru fritted glass crucible. Wash ppt well with at least 7–8 30 ml portions of cold H_2O and dry a 120–140°. Store in desiccator.

(e) *Chloroform soln of aluminum 8-hydroxyquinolate.*—Dissolve Al-oxine in $CHCl_3$ to prep. a 0.5 mg/ml soln. Make fresh each day.

(f) *Sulfuric acid.*—Concd. If a blank detn reveals the presence of F, purify as in **24.45(c)**, diluting and boiling 3 times.

DETERMINATION

Transfer to beaker 150 ml of a liquid or the equivalent quantity of aq. ext. in the case of solids, and add 5 ml 10% K_2SO_4 soln, 10 ml 10% Ba acetate soln, and 3 ml glacial acetic acid and boil. Allow ppt to settle and decant supernatant liquid thru small filter paper. Transfer ppt to filter paper and wash. Transfer paper and ppt to Pt crucible and ignite. Transfer residue to a small porcelain crucible (5 ml or smaller).

Wet a piece of filter paper with the $CHCl_3$ soln of Al oxine in a spot larger in diam. than the top diam. of the crucible and allow to air dry. Add 2 or 3 drops concd H_2SO_4 to ash and place filter paper spot over crucible. Heat crucible covered with the paper to 50–60°C. for 5 min. Observe paper under ultraviolet light. In the presence of F the fluorescence of the Al oxine will be quenched in the area of the spot over the crucible. The limit of identification is ca 0.05 mg F. Run a blank detn on the H_2SO_4.

(3) The following quantitative method for the determination of quaternary ammonium compounds in milk was adopted as first action:

APPARATUS

(a) *Centrifuge.*—Clinical high speed type fitted for 50 ml tubes. An International No. 2 centrifuge with head No. 241 at a speed of 2500 r.p.m. is also satisfactory.

(b) *Centrifuge tubes.*—Heavy wall, 40 ml centrifuge tubes. Pyrex, No. 8400 or equivalent.

(c) *Test tubes.*—Pyrex, glass-stoppered, 15×150 mm.

REAGENTS

(a) *Acetylene tetrachloride.*—Should give a distinct pink color in lower layer after sepn, when 5 ml is shaken for 1 min. with 2 ml of buffer soln, (e), 0.5 ml of eosin yellowish soln, and 5 ml of H_2O soln contg 1 p.p.m. of Cetab, Dobenzyl chloride, Ethyl Cetab, Hyamin 10-X, or laurylpyridinium chloride, or 2 p.p.m. of lauryldimethylbenzylammonium chloride. If the reagent does not meet this test, distil under reduced pressure, rejecting the first 10% of the distillate and collecting ca 80% of the vol. placed in the distn flask.

(b) *Lactic acid soln* (50%).—Add 41 g H_2O to 59 g of lactic acid, 85%, reagent grade and mix.

(c) *Eosin yellowish soln.*—Dissolve 25 mg D&C Red No. 22 in H_2O and dil. to 50 ml.

(d) *Sodium hydroxide soln* (4 *M*).—Dissolve 32 g NaOH in H_2O and dil. to 200 ml.

(e) *Buffer soln* (*p*H 4.5).—Dissolve 25 g citric acid in 75 ml of H_2O and add sufficient (ca 13 ml) of 50% NaOH soln to bring the *p*H to 4.5.

(f) *Aerosol OT soln* (*stock*).—Prep. soln of dioctyl sodium sulfosuccinate to contain 100 mg of the solid chemical per 100 ml.

Det. strength as follows: Pipet 2 ml of a soln contg, in 100 ml, 100 mg of the quaternary ammonium compound to be detd, into glass-stoppered test tube contg 2 ml of acetylene tetrachloride, 2 ml of buffer soln, and 0.5 ml of eosin yellowish soln. Carefully add from buret the Aerosol OT soln in small amounts, shaking mixt. violently for at least 30 sec. after each addn until after sepn into 2 layers only a light pink color is noticeable when test tube is placed against white background. Now continue the addns in 0.01 or 0.02 ml portions until lower layer is no longer pink.

(g) *Standard aerosol OT soln.*—Dil. to 100 ml such a quantity of the stock soln (f) as will produce a soln, 1 ml of which is equivalent to 0.1 mg of the quaternary ammonium compound to be detd. Standardize against a standard soln 1 ml = 0.1 mg of the quaternary to be detd.

DETERMINATION

Pipet 15 ml of acetylene tetrachloride into centrifuge tube; add 6 ml of lactic acid soln and 15 ml of milk to be tested; stopper and shake for ca 3 min. Add 6 ml of NaOH soln and mix carefully until the curd separates thruout the mixt., then shake for at least 30 sec. Centrifuge at high speed (ca 3200 r.p.m.) for 7 min. Decant serum and discard; puncture layer of curd at 2 points and drain the acetylene chloride ext. into a small beaker. Avoiding any drops of the water soln, transfer with pipet 5 ml of the ext. into a glass-stoppered test tube contg 2 ml of buffer soln and 0.5 ml of the eosin yellowish soln; stopper and shake for ca 2 min. Let stand to sep. and observe color of lower layer. If color is faint, place against a white background. If pink color is observed, a quaternary ammonium compound is present. If deep pink or red color is obtained, titrate with the standard Aerosol OT soln. After each addn of the standard soln, shake the mixt. violently for 0.5 to 1 min., let settle into 2 layers and observe the lower one. Continue addns until no pink color is observed in the lower layer when placed against a white background or compared with blank detn. The titration found represents the quantity of quaternary ammonium compound in 5 ml of sample. Calc. to p.p.m.

(4) The following quantitative method for the determination of quaternary ammonium compounds in water solutions was adopted as first action:

APPARATUS (*See* item 3 above)

REAGENTS (*See* item 3 above)

DETERMINATION

Pipet 5 ml of sample into a glass-stoppered test tube contg 2 ml of acetylene tetrachloride, 2 ml of buffer soln, and 0.5 ml of eosin yellowish soln, stopper and shake for 2 min. Let stand to sep. and observe lower layer. If color is faint, examine against a white background. If pink color is observed a quaternary ammonium compound is present. If deep pink or red color is obtained, titrate with the standard Aerosol OT soln as in the method for milk. Calc. to p.p.m.

(5) The following method for the determination of quaternary ammonium compounds as Reineckates, *This Journal*, **35**, 456 (1952), was adopted as first action:

(Applicable to preservatives, tinctures, and isotonic solutions.)

REAGENTS

(a) *Reineckate reagent.*—Place 0.75 g of NH_4 Reineckate in 125 ml Erlenmeyer flask, add 50 ml of H_2O, stopper, shake for ca 2 min., and filter.

(b) *Solvents.*—Reagent grade acetone and alcohol.

DETERMINATION

Place 100 ml of sample contg 10–100 mg of quaternary ammonium compound in 250 ml beaker; add with stirring 5 ml portions of Reineckate reagent until liquid has a bright pink color. Let stand 30 min. and add more reagent unless supernatant liquid has a deep pink color. Stir again for 1–2 min. After several hrs filter thru sintered glass crucible of fine porosity and wash beaker and filter with at least three 15 ml portions of H_2O. (It is unnecessary to transfer all of ppt to crucible.) Wash down sides of crucible with H_2O and dry by suction. If the ppt forms a cake in the filter, mix with the wash H_2O with stirring rod used before.

Dissolve the Reineckate salt in acetone as follows: Set up a suction app. to fit the glass crucible, using as receiver a test tube provided with side tube for application of suction. Using 5 ml pipet, wash down sides of beaker used for pptn and add this liquid to the crucible. Rinse beaker second time and add to liquid in crucible. Stir to dissolve and draw liquid thru with suction. Wash out beaker a third time, and wash down sides of crucible several times with small amounts of acetone. When liquid passing thru is colorless, disconnect, and wash into the test tube with acetone any pink material which may have dried on bottom or outside surface of crucible or on inside of funnel.

Discard the small quantity of greenish solid in crucible which is due to impurities and decomposition products of reagent. Transfer acetone soln to a tared beaker (use 50 ml beaker for 20 mg or less of quaternary ammonium compound and 100 ml beaker for 25 mg or more) and evap. on warm (but not hot) surface. If a few drops of moisture remain, pass gentle current of air into beaker till it appears dry. Dissolve residue by warming in 10 ml (or more if needed) of alcohol; allow solvent to evap. spontaneously, dry in desiccator, weigh and calc. wt of quaternary ammonium compound corresponding to wt of crystals.

(To remove the greenish solid from crucible, add 10–12 ml of HCl (1+1) and stir to dissolve. Draw liquid thru by suction and wash several times with H_2O. Now reverse crucible and wash by filling bottom cavity with solvent. Use two fillings each of H_2O, alcohol, H_2O, acetone, and H_2O in the order given for this purpose.)

28. SPICES AND OTHER CONDIMENTS

(1) The procedure for the preparation of samples of separable types of french dressing, *This Journal*, **35**, 86 (1952), was amended by adding the following paragraph for the preparation of samples in large containers:

For large containers, stir contents of jar thoroly, adding 0.20 g egg albumin powder per 100 g of sample. A mechanical stirrer of the double-beater type is satisfactory. Continue stirring until powder is well dispersed thruout sample. Add sample in portions to Waring blendor and stir each portion ca 5 min. Transfer emulsified portions to jar of similar size as original container. After emulsification, stir entire contents of sample to insure uniform mixt.

Transfer portion of prepd sample to a convenient jar (ca 1 pint). Shake ca 20 times and stir with spatula or spoon ca 20 times before each portion is removed for analysis. Make all weighings immediately after prepn of sample. Correct analytical results for added emulsifier.

29. SUGARS AND SUGAR PRODUCTS

(1) The following method for the determination of the color of raw cane sugars was adopted as first action:

REAGENTS

(a) *Filter aid.*—Celite analytical filter aid (Johns-Manville Corp.).

APPARATUS

(a) *Fractionator.*—Construct fractionator of 35 mm internal diam. heavy wall Pyrex tubing, 145 mm long from top to bottom shoulder where it is sealed to a standard taper stopcock with a 3 mm bore and 9 mm outside diam. tubing. A 55 mm stem is left below the stopcock. Seal a 9 mm outside diam. tube 45 mm long

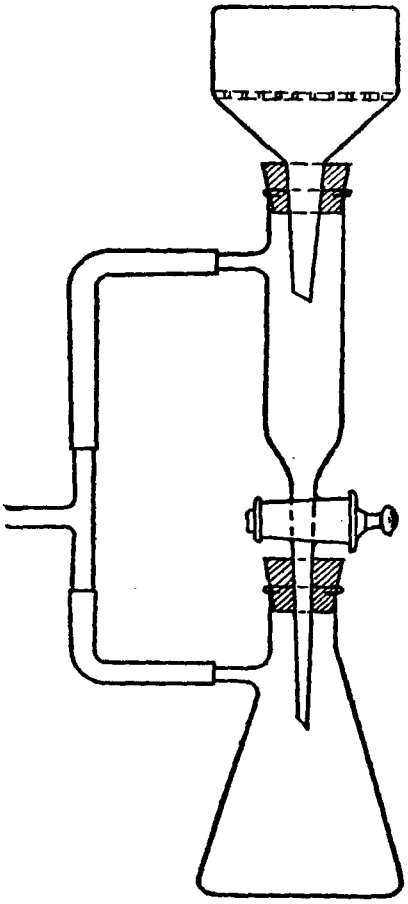

FIG. 1.—Fractionator.

to the body of the fractionator 45 mm below top. Connect to Büchner and source of vacuum thru a "T" tube as shown in Figure 1.

DETERMINATION

Place 60 g of the sugar in a flask, add 40 g of water that has been heated to boiling, and rotate flask until all sugar has been dissolved. Add 3 g of Celite and shake mixt. vigorously for 1 min. Assemble special filtration app. and, with stopcock open, place filter paper, Schleicher and Schuell No. 589 blue ribbon, 7 cm diam., in Büchner funnel, wet down with H_2O and suck excess water by vacuum thru filtering tube into filtering flask. Close stopcock and pour the well shaken mixt. of sugar soln and Celite evenly over filter paper. Carry out filtration at a constant vacuum of 24 inches. Collect ca 10 ml of the first filtrate which is somewhat turbid in the filtering tube and run into filtering flask by opening stopcock. Close stopcock, collect another 10 ml of filtrate in the filtering tube and then run into filtering flask as before to wash inner walls of filtering tube free from any small particles of turbidity. Keep bed of Celite well covered with sugar soln during entire filtration. *Do not allow to run dry.* Collect final clear filtrate in filtering tube, transfer to a small bottle or to a small glass-stoppered Erlenmeyer flask and mix thoroly. Det. refractometer Brix on a portion of the soln, and calc. concn, c, (g dry substance per ml soln) by multiplying Brix by corresponding true density and dividing by 100.

Det. % transmittance at 560 mμ on a spectrophotometer with wavelength and transmittance scales that have been checked, preferably with a standard glass filter supplied by the National Bureau of Standards. Use distd H_2O as the 100% transmittancy standard. The cell thickness used should be such that readings are within the range 25–75% transmittance.

Calc. attenuation index, $a_c = -\log$ transmittance.

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 Enheptin ® (2-amino-5-nitrothiazole) was adopted as first action:

REAGENTS

(a) *Ammonium chloride soln.*—5% aq. soln.

(b) *Boric acid buffer, pH* 9.0.—Prep. the following solns: (1) 6.203 g boric acid and 7.456 g KCl made up to 500 ml with H_2O. (2) 0.2 *M* soln of NaOH. Take 50.0 ml of (1) and 21.40 ml of (2) and make up to 200 ml with H_2O.

(c) *Sodium hydrosulfite soln.*—Prep. 1% soln of Na hydrosulfite in boric acid buffer soln (b) and use not later than 5 min. after prepn.

DETERMINATION

Transfer 2 g ground feed to a 50 ml wide-mouthed volumetric flask, add 10 ml acetone and let stand 2 min., swirling occasionally. Make to vol. with H_2O, mix, and filter immediately thru *coarse* paper. Transfer 25 ml aliquot to 50 ml volumetric flask, add 15 ml of NH_4Cl soln and mix. Make to vol. with H_2O, mix, and filter thru Whatman No. 42 paper (or equivalent), discarding first 10 ml of filtrate.

Place a 4 ml aliquot in each of 2 small beakers. To the first, add 0.5 ml of the freshly prepd 1% Na hydrosulfite soln in boric acid buffer. Make both volumes to 10 ml and read immediately on a spectrophotometer against H_2O at 388.5 mμ. Subtract the reading of the reduced soln from that of the unreduced and compare resulting absorbance to standard curve.

Prep. standard soln by dissolving 100 mg recrystallized 2-amino-5-nitrothiazole in 100 ml acetone and make to vol. of 1 l with H_2O. Transfer aliquots of 4, 8, 12, 16, and 20 ml to 100 ml volumetric flasks and dil. to vol. with H_2O. Treat 5 ml aliquots of each diln as in assay procedure, reading the absorbance of the unreduced soln against the reduced soln as a blank, obtaining readings at 20, 40, 60, 80, and 100 mmg concn.

(2) The following method for *p*-arsanilic acid, applicable in the absence of drugs such as sulfonamides, was adopted as first action:

REAGENTS

(a) *Citric acid soln.*—2.00% aq. soln.

(b) *Sodium nitrite soln.*—Freshly prepd 0.10% aq. soln.

(c) *Ammonium sulfamate soln.*—0.50% aq. soln.

(d) *N*-1-*naphthylethylenediamine dihydrochloride soln.*—0.10% aq. soln. Store in dark bottle.

DETERMINATION

Transfer 4 g ground feed to 200 ml volumetric flask, add 40 ml of citric acid soln and let stand 10 min., swirling occasionally. Add 80 ml of H_2O, then 20 ml of HCl (1+1), mix well, and make to vol. with H_2O. Shake thoroly and filter thru Whatman #42 paper (or equivalent), discarding first few ml if turbid.

To two 50 ml beakers add 5 ml of filtrate and 1 ml of $NaNO_2$ soln. Mix and let stand 5 min. Add 1 ml of ammonium sulfamate soln and let stand 2 min. Then add to the first beaker only, 1 ml of coupling reagent (d), mix and wait 10 min. before making both solns to vol. of 15 ml. Read both against H_2O blank, at 538 mμ in spectrophotometer, subtracting absorbance of feed blank from sample absorbance. Refer to standard graph for concn of drug.

To prep. standard, transfer 0.100 g pure *p*-arsanilic acid to 100 ml volumetric flask, add 0.5 g anhyd. Na_2CO_3, 20 ml H_2O and dissolve. Make to vol. with H_2O. Transfer 10 ml to 100 ml volumetric flask and make to vol. with H_2O. Aliquot 2 ml of second soln to 100 ml volumetric flask and add H_2O to mark. Treat aliquots of 2, 4, 6, and 8 ml in 50 ml beakers with 1 ml of 1+1 HCl, proceeding as in assay, making to final vol. of 15 ml after coupling, and reading at 538 mμ in spectrophotometer, using H_2O blank. Plot absorbance points on graph paper, representing concns of 4, 8, 12 and 16 mmg *p*-arsanilic acid.

(3) The first action method for benzedrine, **32.25** (p. 561), was deleted.

(4) The following methods for the quantitative determination and for the determination of the stereochemical composition of amphetamine in tablets was adopted as first action:

TOTAL AMPHETAMINE

TITRIMETRIC METHOD

Accurately weigh not less than 20 tablets and det. av. wt per tablet. Grind tablets in mortar until the resulting powder will pass an 80 mesh sieve. Accurately weigh sufficient powder to provide 125 mg of amphetamine sulfate (or equivalent of other

amphetamine salt), transfer to 100 ml beaker, add 15 ml H_2O, and stir the mixt. for 15 min. Using rod or policeman transfer as much of the suspension as possible to a fritted glass funnel (medium porosity 40 mm disk is convenient) and filter with suction into a suitable vessel. Break suction and, with portions of H_2O totaling 15 ml, rinse as much adherent material as possible from beaker into funnel. Triturate mixt. in funnel to a uniform paste, and re-apply suction. Make transfer and filtration quant. by repeating the washing 4 addnl times with 10 ml portions of H_2O. With aid of small portions of H_2O, quantitatively transfer filtrate to 100 ml volumetric flask, make to vol. and mix. Transfer a 40 ml aliquot to separatory funnel, add 1 ml 10% NaOH, and ext. with six 25 ml portions of ether. Wash combined ether extracts with two 5 ml portions of H_2O, and ext. the combined washings with two 10 ml portions of ether. Combine ethereal washings with the main ethereal ext., filter thru a pledget of cotton into a 250 ml separatory funnel, rinse separatory funnel which contained the unfiltered ethereal extracts well with ether and pass these rinsings thru the filter so they combine with the filtered ethereal extracts. Ext. filtrate with exactly 20 ml of 0.02 *N* H_2SO_4, and drain the acid extract into 200 ml Erlenmeyer flask. Wash ether with 10, 5, and 5 ml portions of H_2O, combine washings with acid ext. and heat the whole on steam bath until dissolved ether is expelled. Cool and titrate the soln with 0.02 *N* NaOH in presence of methyl red indicator. Calc. % amphetamine sulfate in sample and amount per tablet: 1 ml 0.02 *N* $H_2SO_4 = 3.658$ mg amphetamine sulfate.

CONFIRMATORY GRAVIMETRIC DETERMINATION

In a 250 ml separatory funnel combine the titrated soln with a 50 ml aliquot of the 60 ml of unused aq. ext. remaining in the volumetric flask, acidify by dropwise addition of 0.1 *N* H_2SO_4, and ext. with three 10 ml portions of CCl_4. Discard CCl_4 extracts. To the aq. soln, which has been sepd from as much CCl_4 as practicable, add 4.10 g $NaHCO_3$ and swirl funnel until salt has mostly dissolved. Rapidly introduce into funnel 1.0 ml acetic anhydride, A.C.S. reagent grade, by blowing reagent in from a 1 ml pipet. Immediately stopper funnel securely and shake vigorously until evolution of CO_2 has nearly ceased (release pressure in funnel frequently during shaking by opening stopcock). Add another 1.0 ml portion of anhydride, and continue to shake funnel until evolution of CO_2 has ceased (5–10 min. after addn of second portion of anhydride). Allow mixt. to stand 5 min. and completely ext. the acetylamphetamine by shakeouts with 50 ml portions of $CHCl_3$ (4 should be sufficient; test for complete ext. with a 5th shakeout). Filter extracts thru pledget of cotton, rinse filter with $CHCl_3$, conc. filtrate to a small vol. on steam-bath in current of air, quantitatively transfer concentrate to tared 50 ml beaker by rinsing with small portions of $CHCl_3$, and continue the concentration until solvent is removed. Heat residue of acetylamphetamine in an oven (not forced-draft type) at 80° for 1 hr, cool in desiccator and weigh. Calc. % amphetamine salt in sample and amount per tablet: Acetylamphetamine × 1.0395 = amphetamine sulfate.

Induce crystallization in the residue, if it has not crystallized spontaneously, by trituration, adding a small seed crystal of racemic amphetamine if necessary. Powder the crystalline derivative finely and mix well. The pure acetyl derivative of *d*-amphetamine melts at 124.5–125°C.; that of the racemic substance at 93–93.5°C.

STEREOCHEMICAL COMPOSITION OF TOTAL AMPHETAMINE

POLARIMETRIC METHOD

Accurately weigh 90 mg of the derivative, transfer quantitatively to 5 ml volumetric flask, and make to vol. with U.S.P. $CHCl_3$. Det. optical rotation of soln in

a semi-micro 2 dm tube (bore ca 4.5 mm, vol. 3–4 ml) at the same temp. at which the soln was made to vol. Acetyl-*d*-amphetamine is levorotatory in $CHCl_3$.

In measuring rotation with polariscope, take 10 readings on soln and calc. the av. to 0.001°. In the same way, det. the av. reading with same tube filled with U.S.P. $CHCl_3$, and use the av. zero-point reading thus obtained to correct the av. reading given by the soln.

If saccharimeter is used instead of polariscope, estimate all readings to 0.05 division, calc. the av. to 0.01 division, correct for zero-point, and multiply value so obtained by 0.3468 to obtain rotation, α, in angular degrees.

Calc. the specific rotation, $[\alpha]$, to 0.1° by the equation

$$[\alpha]=\frac{100\,\alpha}{c\times l}$$

in which c is concn of acetylamphetamine in g/100 ml and l is length of tube in decimeters.

Determine the % *d*-amphetamine by means of the equation $\%\, d=50+(50[\alpha]/44)$ in which $[\alpha]$ is the specific rotation of the acetyl derivative from the sample, 44 is the specific rotation of pure acetyl-*d*-amphetamine, and in which the sign of rotation has been ignored.

CONFIRMATORY THERMAL ANALYSIS

In a melting point tube having an internal diam. of 2–3 mm at the bottom and a length of ca 70 mm, place sufficient finely powd. acetyl-*dl*-amphetamine (ca 8 mg) to form column 5 to 6 mm high after tube and contents have been tapped firmly several times on a hard surface.

Select a thermometer the range of which includes temperatures between 90 and 130°C. and whose graduations will permit readings to 0.5° with the aid of a low-power hand lens. An Anschütz-type thermometer is convenient but not necessary. The thermometer need not be calibrated, but if not, it is important that the same thermometer be used in determining the standard m.p. curve and the m.p. of the derivative from the sample.

Fix melting point tube securely to the thermometer by two small rubber bands, one of which should be placed near the top of m.p. tube and the other as far down as possible without allowing liquid* in bath to touch the band. Suitable bands may be cut from rubber tubing of the proper size. Adjust m.p. tube so that the middle of column of specimen coincides approximately with middle of thermometer bulb.

Support assembly in a mechanically-stirred m.p. bath. Raise temp. of bath rapidly until it is about 5° below anticipated m.p. (temp. at which specimen becomes entirely a clear liquid), then regulate heating carefully so that the rise in temp. does not exceed 0.5° per min. After specimen begins to melt stir continuously with a chromel wire (0.4 mm diam.; flatten the lower end for a distance of ca 3 mm. and bend flattened portion at a right angle ca 1 mm from tip so as to form a hoe-like stirrer) while inspecting it carefully with ca 10 power hand lens. Note temp. at which the last crystalline material disappears and record it as the m.p. Remove tube and thermometer from bath, induce the melt to solidify by stirring (seeding if necessary) and repeat detn. Replicate detns will not differ by more than 1° if carefully carried out.

Following the same procedure, det. m.p. of pure acetyl-*d*-amphetamine.

Prep. a series of standard mixtures of acetyl-*d*- and acetyl-*l*-amphetamine having the following compositions expressed in mg: 80 *d*+20 *dl*, 60 *d*+40 *dl*, 40 *d*+60 *dl*, 20 *d*+80 *dl*. These mixtures contain, resp., 90%, 80%, 70%, and 60% of *d*-isomer.

* DC 200 Silicone fluid, viscosity grade 20 centistokes at 25°C., Dow Corning Corporation, Midland, Michigan, is convenient for a bath liquid.

In each case accurately weigh out each component into small (18×55 mm) test tube, and hold tube in bath heated to 130–135°C. until contents have completely melted. Stir molten contents with small stainless steel spatula until well mixed, then withdraw tube from bath and continue to stir until melt has completely solidified. Transfer solidified material as completely as possible to small mortar, powder finely and mix thoroly. Det. m. p. of each mixt. in the manner described above. In each mixt. the beginning of fusion (softening, appearance of liquid phase) will be noted at ca the same temp. (ca 93°), but the temp. at which the system becomes entirely liquid (m.p.) will depend on the composition of the mixt. Unlike in the m.p. detns of pure *dl*- and *d*-derivatives, it is not important to stir the mixtures continuously after the first evidence of fusion. After considerable liquid phase has formed, the specimen should be stirred occasionally as the solid phase diminishes in size. Stirring should be continuous during ca the last 2 min. of the detn, i.e., during inspection in anticipation of the disappearance of the last portion of crystalline matter. Any solid matter adhering to walls of tube above melt should be pushed down into melt by the wire stirrer.

On coordinate paper, plot the av. m.p. (ordinate) of each specimen against the composition (abscissa) expressed as % acetyl-*d*-amphetamine, and draw a smooth curve which is in best conformity with the 6 plotted points.

Det. m.p. of the derivative obtained from the tablets, and, by referring to the standard curve, estimate the % *d*-isomer present.

(5) The following method for the determination of sulfadiazine in the presence of other sulfonamides, and of sulfamerazine and sulfadiazine in mixtures of these two compounds, was adopted as first action:

SULFADIAZINE AND SULFAMERAZINE

REAGENTS

(a) *Citrate buffer soln.*—37 g Na citrate dihydrate and 32 ml concd HCl dild to 250 ml with H_2O.

(b) 2-*Thiobarbituric acid soln.*—Recrystallize twice from hot H_2O. Dissolve 5 g of this recrystallized acid in 5 ml 4 *N* NaOH dild with 500 ml H_2O. Add 250 ml citrate buffer soln and adjust *p*H to 2.0 to obtain final reagent. Reagent is stable when stored in glass-stoppered bottle in refrigerator.

DETERMINATION

Prep. sample soln contg ca 0.1 g mixed sulfonamides by intermittant shaking with 50 ml *N* HCl during a 10 min. period. Filter, if necessary, to obtain a clear soln and dil. filtrate and washings to 100 ml. To 5 ml of this soln add 7.5 ml *N* HCl and dil. to 100 ml. (The soln obtained should contain ca 5 mg mixed sulfonamides per 100 ml 0.1 *N* HCl). To 1.0 ml of this soln in a glass-stoppered test tube add 10.0 ml of buffered 2-thiobarbituric acid soln **(b)**. Stopper and heat 1 hr at 100°C. Weigh tubes before and after heating and compensate for any loss of moisture during heating by addn of H_2O. Treat similarly a 1.0 ml standard contg 25 mmg sulfadiazine in 0.1 *N* HCl and a 1.0 ml sample of acid for a blank. Det. absorbancies at 532 mμ relative to the blank and calc. the sulfadiazine content of sample.

Det. the absorbancies at 305 mμ of the prepd soln and of sulfadiazine and sulfamerazine standards each contg 5.0 mg pure sulfonamide per 100 ml 0.1 *N* HCl, relative to the blank acid. Subtract from sample value absorbance due to the sulfadiazine content and calc. quantity of sulfamerazine in sample. The ultraviolet spectrum of the sample should be examined to assure absence of background interference.

TABLE 1.—*Optical-crystallographic properties of some crystalline drugs*[a]

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Antihistamines								
Antergan Hydrochloride	1.587	1.635	1.734	+				Short prisms. Inclined figs.
Anthallan Hydrochloride	1.505	1.585	1.617		p	−		Small rods & irregular fragments. No figs.
Bromothen Hydrochloride	1.617	1.654	1.734		i	+		Very small rods. Inclined figs. occas'l
Chlorcyclizine Monohydrochloride (Di-Paralene HCl)	1.590	1.610	1.665	+				Thin platy fragments. Op. Ax. figs. common
Chlorcyclizine Dihydrochloride (Perazil Dihydrochloride)	1.610	1.660	1.665	−	p	+	very small	Short rods & thin 6-sided plates. Op. Ax. figs. common
Chlorprophenpyridamine Maleate (Chlor-Trimeton Maleate)	1.533	n_i 1.668	sl < 1.734					Box-like prisms & irregular fragments. Figs. infrequent
Chlorothen Citrate (Tagathen)	1.583	1.603	1.645					Minute plates & shreds. Op. Ax. figs. rare
Chlorothen Hydrochloride	1.553	1.625	>1.734					Massive fragments, some rectangular Op. Ax. figs. occas'l
Dimenhydrinate (Dramamine) (Unsatisfactory for optical-crystallographic study.)								Platy mat'l & rods
Diphenhydramine Hydrochloride (Benadryl HCl)	1.602	1.625	1.630	−	p	−		6-sided plates.
p-Fluorobenzyl (D.P.E.) Hydrochloride	1.585	1.600	1.668	+	p	−	large	Rods & square plates. Op. Ax. figs. occas'l
Linadryl Hydrochloride	1.577	1.631	1.672	−	p, i	+		Elongated 6-sided & irregular fragments. Figs. rare
Methaphenilene Hydrochloride (Diatrine HCl)	1.604	1.675	1.733	−				Elongated 6-sided rods with obtuse ends. Op. Ax. figs. frequent
Methapyrilene Hydrochloride (Histadyl, Thenylene HCl)	1.588	1.654	>1.695 but <1.734	−				Thick hexagonal plates. Inclined Op. Ax. figs. frequent
Phenergan Hydrobromide	1.667	1.675	>1.733	+			small	Massive prisms, Elongated or short & stubby
Phenergan Hydrochloride	1.617	1.691	1.733	−	p	+		Rods & irregular fragments
Pyrilamine Maleate (Unsatisfactory for optical-crystallographic study.)								Rods & irregular fragments
Pyrrolazote Hydrochloride	1.690		1.737					Stout prismatic forms. No figs.

[a] Abbreviations: Bx. Ac. = Acute bisectrix; Bx. Ob. = Obtuse bisectrix; fig. = figure: i = inclined; n_i = intermediate index; Op. Ax. = Optic Axis; p = parallel; s = symmetrical.

TABLE 1—(*continued*)

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Thenfanil Hydrochloride	1.590		1.680				large	Square plates & stubby prisms. Inclined figs. common
Thonzylamine Hydrochloride (Neohetramine HCl)	1.612	1.679	1.691	−	p	+		Rods & platy material
Tripelennamine Hydrochloride (Pyribenzamine HCl)	1.580	1.655	1.705	−				Rectangular plates & prisms from water. Op. Ax. figs. common
Barbiturates								
Allylbarbituric acid (5-allyl-5-isobutylbarbituric acid)	1.508	n$_i$1.521	1.577	+	p	+	moderate	Rods and plates
Alphenal (5-allyl-5-phenylbarbituric acid)	1.551	1.578	1.645	+	p	−	67°	Op. Ax. fig. common
Alurate (5-allyl-5-isopropylbarbituric acid)	1.522	—	1.602					Both n's common
Amytal (5-isoamyl-5-ethylbarbituric acid)	1.467	—	1.539		p	+		Both n's common
Amytal Sodium	(n) 1.505	—	—	Isotropic				
Barbital (diethylbarbituric acid)	1.445	1.548	1.580					All n's common
Barbital Sodium	1.512		1.615			+		Both n's common
Butallylonal [5-(2-bromallyl)-5-sec-butylbarbituric acid]	1.524	1.577	1.603	−	p	+	moderate	Rosettes of tiny rods and blades. Bx. Ac. figs. occas'l
Butethal (5-butyl-5-ethylbarbituric acid)	1.454	1.518	1.556	−	i		large	Rods & needles. Op. Ax and Bx. Ac. figs. common
Cyclobarbital (Phanodorn) (5-ethyl-5-cyclohexenylbarbituric acid)	1.515	1.546	1.621	+		±	69°	Bx. Ac. & Bx. Ob. figs. common
Cyclopal (5-cyclopentenyl-5-allylbarbituric acid)	1.520	1.575	1.626	−		−	85°	Bx. Ac. fig. common
Delvinal (5-ethyl-5-(1-methyl-1-butenyl) barbituric acid)	1.506	1.544	1.672	+	p	−	61°	Bx. Ac. fig. common
Dial (5,5-diallylbarbituric acid)	1.516	1.572	1.625	−	s		large	Op. Ax. fig. common
Hexethal (Ortal) (5-ethyl-5-*n*-hexylbarbituric acid)	1.473	1.519	1.549	−		−	76°	Bx. Ac. fig. common
Hexobarbital (Evipal) (5-cyclohexenyl-1,5-dimethylbarbituric acid)	1.546	1.608	1.634	−	p	+	64°	Bx. Ac. & Op. Ax. figs. common
Mephobarbital (Mebaral) (5-ethyl-l-methyl-5-phenylbarbituric acid)	1.594	1.610	1.651	+	p	−	65°	Bx. Ac. fig. common
Pentobarbital [5-ethyl-5-(1-methylbutyl) barbituric acid]	1.465	—	1.565	−	i		very large	———
Pentobarbital Sodium	1.477	—	1.523					———
Phenobarbital (5-ethyl-5-phenylbarbituric acid)	1.557	1.620	1.667		p	−		β very common

TABLE 1—(*continued*)

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Phenobarbital Sodium (unstable)								
Secobarbital (Seconal) [5-allyl-5-(1-methylbutyl barbituric acid]	1.487	1.557	1.563	−	p	+	31°	Bx. Ac. fig. common
Secobarbital Sodium	1.490	n_i1.500	1.525					n_i & γ common
Sigmodal [5-*s*-amyl-5-β-bromallylbarbituric acid]	1.519	1.583	1.634	−		+	80°	Bx. Ac. fig. common
Sulfonamides								
Sulfacetamide	1.559	1.564	1.727	+	s		21°	
Sulfadiazine	1.596	1.675	1.830	+	p, i	±	76°	
Sulfadiazine[c]	1.615	1.663	>1.734		p	±		Rods
Sulfaguanidine	1.606	1.663	1.734					Op. Ax. fig.
Sulfaguanidine monohydrate	1.586	1.649	1.731	+	p, i	±	86°	
Sulfallantoin (sulfanilamide+allantoin—addition product)	1.513	1.590	>1.690 <1.733					Op. Ax. fig.
Sulfamerazine	1.568	1.657	1.687	−	p	±	58°	Bx. Ac. fig. all n's common
Sulfamerazine[c]	1.587		1.675					
Sulfamethazine	1.584	1.623	1.778					Rods
Sulfamidazole (sulfanilamide+sulfathiazole–double crystal[b]	1.661	1.678	>1.733	+	p	−	small	Bx. Ac. fig.
Sulfanilamide phase B (anhyd.)	1.555	1.672	1.85	+	p	−		Stable form coml prepns
Sulfanilamide HCl	1.540	1.655	1.690		p	−		Rods
Sulfapyridine, Phase I	1.670	1.736	1.813	+	p, i	±	88°	Tabular to equant stable form coml prepns
Sulfapyridine[c]	1.680	1.733	>1.733					Op. Ax. fig.
Sulfapyridine Sodium monohydrate	1.590		1.700		p	−		
Sulfasuxidine	1.578	1.676	1.710	−	i		58°	Rods
Sulfathiazole, Phase I	1.674	1.685	>1.733	+			small	α & β common
Sulfathiazole, Phase II	1.598	1.741	1.780	−	p, i	±	52°	Lath shaped
Sulfathiazole[c]	1.695	n_i1.733	>1.733					
Sulfathiazole Sodium sesquihydrate	1.596		1.621					
Sympathomimetic Amines								
d-Amphetamine Hydrochloride (Dexedrine HCl)	1.560	1.592	1.622	+	p, i		very large	Large plates & rods. Op. Ax. figs. occas'l
dl-Amphetamine Phosphate, dibasic	1.549	1.589	1.655	+			large	Small platy crystals. Bx. Ac. figs. common

[b] Equimolecular proportions.

[c] The second set of optical properties in each case represents intermediate data which are quite commonly found in some commercial samples. They probably represent a hydrous form or merely a different common orientation of the crystal.

TABLE 1—(*continued*)

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
d-Amphetamine Sulfate (Dexedrine SO_4)	1.505	1.545	1.608	+		−	small	6–8-sided plates
dl-Amphetamine Sulfate (Benzedrine SO_4)	1.520	1.535	1.580	+		−	very sm.	
Benzazoline Hydrochloride (Priscoline HCl)	1.586	1.604	1.703	+	i			6-sided plates. Inclined Op. Ax. figs. common
d-Desoxyephedrine Hydrochloride (Methamphetamine HCl)	1.530	1.537	1.615	+				Irregular fragments Op. Ax. figs. frequent
dl-Desoxyephedrine Hydrochloride	1.535		1.620		p	−		Small 6-sided platy or rod-like crystals. No figs.
dl-Ephedrine Hydrochloride (Racephedrine HCl)	1.570	1.608	1.630	−				Irregular fragments. Occasional Op. Ax. figs.
l-Ephedrine Hydrochloride	1.530	1.603	1.638	−	p	−	70°	Elongated prisms & rods
l-Ephedrine Sulfate	1.540	1.565	1.587	+	p		large	6-sided plates & rods
dl-Epinephrine	1.551	1.599	1.736	+	p	−	moderate	Thin, blade-like, 6-sided crystals in rosettes. Bx. Ac. figs. common
l-Epinephrine	1.551	1.599	1.736	+	p	−	moderate	Rosettes of thin, blade-like, 6-sided crystals. Bx. Ac. figs. common
Hydroxyamphetamine Hydrobromide (Paredrine HBr)	1.560	1.680	1.734	−				Irregular fragments. Inclined Op. Ax. fig. frequent
Naphazoline Nitrate	1.560	1.619	>1.740	+	s, i			6-sided plates & irregular fragments. Bx. Ac. figs. common
Phenylpropylmethylamine Hydrochloride (Vonedrine HCl)	1.577		1.603		p	−		Small rod-like fragments. No figs.
Supriphen Hydrochloride	1.507	1.604	1.668	−	p	±		Rectangular rods. Bx. Ob. figs. common
dl-Synephrine base (Desoxyepinephrine)	1.546	1.604	1.725	+	s, i		large	Platy crystals, often diamond-shaped. Op. Ax. figs. common
Synephrine Hydrochloride	1.549	1.605	1.664	+	p, i		large	Large plates. Bx. Ac. figs. frequent
dl-Synephrine (+) Tartrate (Neutral Salt)	1.516	n_i1.620	1.689	+	i	+	large	Rods & plates. Partial Op. Ax. figs. common
Tuaminoheptane Sulfate (Tuamine Sulfate)	1.458_ω		1.468_ϵ	+	p			Irregular-shaped plates & fibrous flakes. Figs. frequent
Veritol Sulfate (Isodrine Sulfate)	1.516	1.552	1.645	+	s	+		Rhombohedral or 6-sided plates

TABLE 2.—*Determinative table for drugs, arranged according to ascending value of the lowest index*[a]

α	β	γ	COMPOUND
		Antihistamines	
1.505	1.585	1.617	Anthallan Hydrochloride
1.533	n_i1.668	sl <1.735	Chlorphenpyridamine Maleate (Chlor-Trimeton Maleate)
1.553	1.625	>1.734	Chlorothen Hydrochloride
1.577	1.631	1.672	Linadryl Hydrochloride
1.580	1.655	1.705	Tripelennamine Hydrochloride (Pyribenzamine HCl)
1.583	1.603	1.645	Chlorothen Citrate (Tagathen)
1.585	1.600	1.668	p-Fluorobenzyl (D.P.E.) Hydrochloride
1.587	1.635	1.734	Antergan Hydrochloride
1.588	1.654	>1.695 but <1.734	Methapyrilene Hydrochloride (Histadyl, Thenylene HCl)
1.590	1.610	1.665	Chlorocyclizine Monohydrochloride (Di-Paralene HCl)
1.590		1.680	Thenfanil Hydrochloride
1.602	1.625	1.630	Diphenhydramine Hydrochloride (Benadryl HCl)
1.604	1.675	1.733	Metaphenilene Hydrochloride (Diatrine HCl)
1.610	1.660	1.665	Chlorcyclizine Dihydrochloride (Perazil di-HCl)
1.612	1.679	1.691	Thonzylamine Hydrochloride (Neohetramine HCl)
1.617	1.654	1.734	Bromothen Hydrochloride
1.617	1.691	1.733	Phenergan Hydrochloride
1.667	1.675	>1.733	Phenergan Hydrobromide
1.690		1.737	Pyrrolazote Hydrochloride
		Barbiturates	
1.445	1.548	1.580	Barbital (Diethylbarbituric acid)
1.454	1.518	1.556	Butethal (Neonal)
1.465		1.565	Pentobarbital (Nembutal)
1.467		1.539	Amobarbital (Amytal)
1.473	1.519	1.549	Hexethal (Ortal)
1.476	1.573	1.610	Probarbital (Ipral)
1.477		1.523	Pentobarbital Sodium (Nembutal Sodium)
1.487	1.557	1.563	Secobarbital (Seconal)
1.490	n_i1.500	1.525	Secobarbital Sodium (Seconal Sodium)
(n) 1.505	Isotropic		Amobarbital Sodium (Amytal Sodium)
1.506	1.544	1.672	Vinbarbital (Delvinal)
1.508	n_i1.521	1.577	Allylbarbituric Acid (Sandoptal)
1.512		1.615	Barbital Sodium
1.515	1.546	1.621	Cyclobarbital (Phanodorn)
1.516	1.572	1.625	Diallylbarbituric Acid (Dial)

TABLE 2—(*continued*)

α	β	γ	COMPOUND
1.519	1.583	1.634	Sigmodal
1.520	1.575	1.626	Cyclopal
1.552		1.602	Aprobarbital (Alurate)
1.524	1.577	1.603	Butallylonal (Pernoston)
1.546	1.608	1.634	Hexobarbital (Evipal)
1.551	1.578	1.645	Alphenal
1.557	1.620	1.667	Phenobarbital (Luminal)
1.594	1.610	1.651	Mephobarbital (Mebaral)
		Sulfonamides	
1.513	1.590	>1.690 but <1.733	Sulfallantoin
1.540	1.655	1.690	Sulfanilamide HCl
1.555	1.672	1.85	Sulfanilamide, Phase B (anhydrous)
1.559	1.564	1.727	Sulfacetamide
1.568	1.657	1.687	Sulfamerazine
1.578	1.676	1.710	Succinylsulfathiazole (Sulfasuxidine)
1.584	1.623	>1.778	Sulfamethazine
1.586	1.649	1.731	Sulfaguanidine Monohydrate
1.587		1.675	Sulfamerazine
1.590		1.700	Sulfapyridine Sodium monohydrate
1.596		1.621	Sulfathiazole Sodium sesquihydrate
1.596	1.675	1.830	Sulfadiazine
1.598	1.741	1.780	Sulfathiazole, Phase II
1.606	1.663	1.734	Sulfaguanidine
1.615	1.663	>1.734	Sulfadiazine
1.661	1.678	>1.733	Sulfamidazole
1.670	1.736	1.813	Sulfapyridine, Phase I
1.674	1.685	>1.733	Sulfathiazole, Phase I
		Sympathomimetic Amines	
1.458_{ω}		1.468_{ϵ}	Tuaminoheptane Sulfate (Tuamine Sulfate)
1.505	1.545	1.608	d-Amphetamine Sulfate (Dexedrine SO_4)
1.507	1.604	1.668	Supriphen Hydrochloride
1.516	1.552	1.645	Veritol Sulfate (Isodrine Sulfate)
1.516	n_i1.620	1.689	dl-Synephrine (+) tartrate (Neutral Salt)
1.520	1.535	1.580	dl-Amphetamine Sulfate (Benzedrine SO_4)
1.530	1.537	1.615	d-Desoxyephedrine Hydrochloride (Methamphetamine HCl)
1.530	1.603	1.638	l-Ephedrine Hydrochloride
1.535		1.620	dl-Desoxyephedrine Hydrochloride
1.540	1.565	1.587	l-Ephedrine Sulfate
1.546	1.604	1.725	dl-Synephrine base (Desoxyepinephrine)
1.549	1.589	1.655	dl-Amphetamine Phosphate, dibasic
1.549	1.605	1.664	Synephrine Hydrochloride

TABLE 2—(*continued*)

α	β	γ	COMPOUND
1.551	1.599	1.736	dl-Epinephrine
1.551	1.599	1.736	l-Epinephrine
1.560	1.592	1.622	d-Amphetamine Hydrochloride (Dexedrine HCl)
1.560	1.619	>1.740	Naphazoline Nitrate
1.560	1.680	1.734	Hydroxyamphetamine Hydrobromide (Paredrine HBr)
1.570	1.608	1.630	dl-Ephedrine Hydrochloride (Racephedrine HCl)
1.577		1.603	Phenylpropylmethylamine Hydrochloride (Vonedrine HCl)
1.586	1.604	1.703	Benzazoline Hydrochloride (Priscoline HCl)

[a] See Table 1, p. 110, for symbols.

SULFADIAZINE IN THE PRESENCE OF OTHER SULFONAMIDES

Det. sulfadiazine as directed above from a prepd soln contg ca 25 mmg sulfadiazine per ml 0.1 *N* HCl.

(6) The first action gravimetric method for the determination of ketosteroids, *This Journal*, **34**, 81–83 (1951), and the first action colorimetric method for the determination of ketosteroids, *This Journal*, **34**, 83–85 (1951), were revised as follows:

(a) Combine the two determinations into a single method by deleting item (7), first two lines: "The following method . . . first action." and changing the word "(gravimetric)" in the last two lines of page 81 to "(colorimetric)."

(b) On page 83 delete the last two sentences "Dry in a vacuum . . . procedure." and substitute the sentence "Weigh (residue may be dried in a vacuum desiccator for a semiquantitative estimate of ketosteroid weight) and prep. an alc. soln of the ketosteroids from the residue to contain 90–120 mmg ketosteroids per ml."

(c) On page 84 delete the title "Sample Solution" and the accompanying sentence.

(7) The accompanying tables (pp. 110–116) for the identification of drugs by their microscopic crystallographic properties were adopted as first action.

33. COSMETICS

(1) The following first action methods were adopted as official:

(a) Urea in deodorants, *This Journal*, **34**, 89 (1951).

(b) Chlorides in deodorants, *This Journal*, **34**, 90 (1951).

(c) Sulfates in deodorants, *This Journal*, **34**, 90 (1951).

(d) Methenamine, *This Journal*, **35**, 90 (1952).

(e) Phenolsulfonates, *This Journal*, **35**, 91 (1952).

(f) Qualitative and quantitative methods for the examination of thioglycolate solutions, *This Journal*, **35**, 91 (1952).

(g) Qualitative tests for potassium bromate and sodium perborate, *This Journal*, **35**, 92 (1952).

34. COLORING MATTERS

(1) The following first action methods were adopted as official:

(a) Intermediates derived from phthalic acid, *This Journal*, **34,** 91–93 (1951).

(b) Sulfonated amine intermediates: Lake Red C Amine in D&C Red Nos. 8 and 9, *This Journal*, **35,** 92 (1952).

(c) Subsidiary and lower sulfonated dyes: lower sulfonated dyes in FD&C Yellow No. 5, *This Journal*, **35,** 93 (1952).

(d) Ether extracts, **34.52** and **34.54–34.58** (pp. 679–680).

(2) The first action method, *This Journal*, **34,** 93 (1951), for pseudocumidine in FD&C Red No. 1, was deleted, first action.

(3) The following method for the determination of higher sulfonated dye in FD&C Yellow No. 6 was adopted as first action:

REAGENTS

(a) *Isoamyl alcohol.*—Reagent grade.

(b) *Hydrochloric acid* (1+25).—Dil. 40 ml concd HCl to one l with distd H_2O.

APPARATUS

(a) *Spectrophotometer.*—Suitable for measuring absorbance at 450–500 mμ.

(b) *Absorption cells.*—Preferably 5 cm path length.

EXTRACTION

Dissolve 100 mg of the FD&C Yellow No. 6 in 100 ml 1+25 HCl. Dil. 10 ml of this soln with 40 ml 1+25 HCl and ext. by shaking the soln successively in 5 separatory funnels, each contg 50 ml isoamyl alcohol. Transfer acid layer to a 100 ml volumetric flask. Wash amyl alcohol extracts with two 25 ml portions of 1+25 HCl, passing each portion thru the funnels in the same order as used for original extn. Add washings to the acid soln of subsidiary and dil. to 100 ml with distd H_2O. Det. absorbance of extd soln at the max. Det. absorbance per mg/l of a standard soln of the subsidiary in (1+25) HCl, at the same wave length and in the same cell.

$$\textit{Calculation:}\ \frac{\text{Absorbance of extract}}{\text{Absorbance per mg/l of standard}} = \%\ \text{subsidiary}$$

35. EXTRANEOUS MATERIALS: ISOLATION

(1) The first action method for the sediment test on milk, **35.9** (p. 705), as revised, *This Journal*, **35,** 99 (1952), **36,** 87 (1953), was further revised as follows and adopted as first action:

(a) Insert as section **35.9(c)**:

Sediment filtering apparatus.—App. must hold $1\frac{1}{4}''$ sediment disk and have effective filtering area $1\frac{1}{8}''$ in diam. This $1\frac{1}{8}''$ area must be unobstructed except for wire screen or wire screen and perforated plate support for filter disk. App. should be supported in filter flask so vacuum can be used for rapid filtration or flask air outlet closed to stop filtration. App. should have ca 80° funnel with min. capacity of 80 ml and max. capacity of 450 ml. Test app. by filtering H_2O suspension of C thru standard disk. Disk should have clean, sharply defined border. When sediment

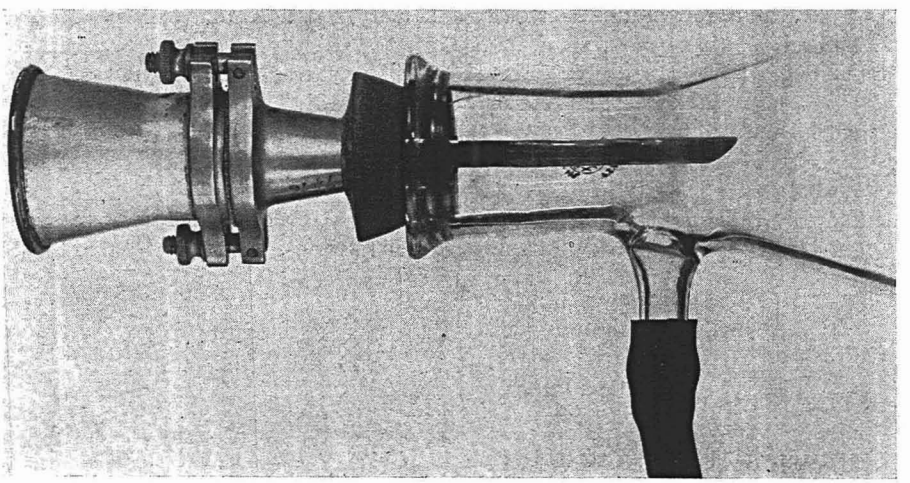

FIG. 2.—Sediment filtering apparatus, assembled.

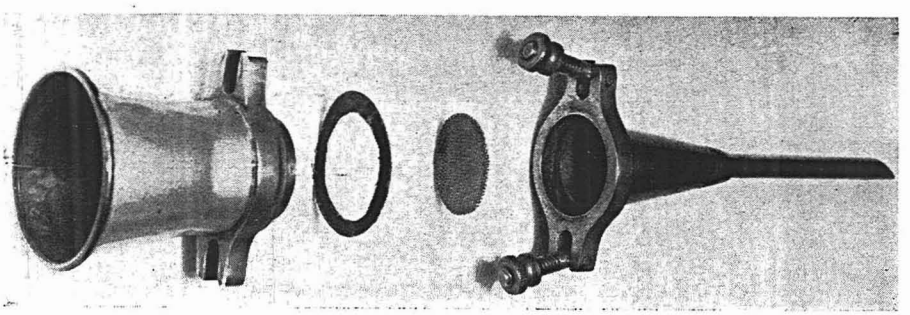

FIG. 1.—Sediment filtering apparatus, unassembled.

suspension is filtered, sediment should be evenly distributed over disk with no pattern formation. Figures 1 and 2 are photographs of a suitable app.

(b) The present section 35.9(c) was changed to 35.9(d) and the sentence beginning at the eighth line of the third paragraph, "Mix thoroly and pass mixture thru standard sediment disk in filtering device having filtering area measuring 1¾" in diameter." was changed to: "Mix thoroly and pass mixture thru standard sediment disk in filtering app. (c)."

(c) The present section 35.9(d) was changed to 35.9(e) with the following changes:

In the next to the last line of the first paragraph and in the first and last lines of the second paragraph, "(c)" should be changed to "(d)."

In the second line of the second paragraph, "(c)" should be inserted after "apparatus."

In the seventh line of the second paragraph, "funnel" should be changed to "apparatus (c)."

(d) The present section 35.9(e) was changed to 35.9(f) with the following changes.

In the fourth line "(c)" should be changed to "(d) or (e)."

Insert just before the last sentence of this paragraph, "The standards that more nearly resemble the test disk being graded should be used in each case."

(2) The following method for the determination of heavy and light filth in granulated nut meats was adopted as first action to replace method 35.22(c) (p. 711) which was deleted:

Weigh 100 g of sample into 400 ml beaker. Cover nutmeats with petr. ether, cover beaker and boil gently 30 min. Add sufficient ether to maintain the original vol. Decant ether thru 7 cm filter paper on Büchner funnel. Add ca 200 ml $CHCl_3$ to beaker and allow to settle 10–15 min. Remove floating nutmeats with spoon to 15 cm filter paper in Büchner funnel. Decant $CHCl_3$ thru 15 cm filter paper, being careful not to disturb any heavy residue in bottom of beaker. Repeat extn with small quantities of $CHCl_3$-CCl_4 (1+1) mixt. until heavy residue becomes relatively free of nutmeat particles. Transfer residue, if any, from beaker to ashless filter paper and examine for heavy filth. Ignite and weigh to det. sand and dirt.

Transfer nutmeats to smooth sheet of paper and retain 15 cm filter paper to be rinsed later as directed. Dry overnight at room temp. or in an oven at ca 80° for 1 hr. Place dry nutmeats in a 2 l Wildman trap flask, assisting the transfer and rinsing the 15 cm filter paper with 300 ml 60% alcohol. Stir slightly and allow mixt. to soak 10 min. Add 250 ml Tween 80*—60% alcohol soln (40 ml Tween 80+210 ml 60% alcohol) and mix. Add 75 ml gasoline. Quickly add 5 g of powdered tetrasodium salt of ethylenediamine tetraacetic acid to 250 ml 60% alcohol and, before completely dissolving, add to trap flask. Immediately stir for 2 min. in the usual manner to mix in gasoline. Fill flask with 60% alcohol. (Add reagents, mix gasoline, and fill flask with 60% alcohol without time interruption. Operate only one flask at a time during these steps.) Stir occasionally during only the first 15 min. after the flask is filled. Allow flask to stand strictly undisturbed 1 addnl hr. Trap off and filter using only 60% alcohol as a rinse. Repeat the above extn using 40 ml gasoline and 1½ hours standing. Examine filters microscopically.

(3) The first procedure in section 35.22(b) for light filth in shelled nuts beginning "Weigh 100 g into suitable beaker, . . . " and ending at "Trap off, filter, and examine microscopically." was deleted.

* Atlas Powder Company, Wilmington, Delaware.

(4) The first action method for light filth in Spanish peanuts, *This Journal* 35, 94 (1952), was designated as "Light filth in peanuts with adhering testa."

(5) The first action sieving method for insect fragments and rodent hairs in baked products, prepared cereals, and alimentary pastes, **35.28(a)** (p. 714) was revised to read as follows and adopted as first action:

Weigh 225 g of sample into a 2 l beaker and add sufficient hot H_2O to soften and sat. the material. If lumps persist or if H_2O is not immediately absorbed uniformly thru the entire mass (*e.g.*, in the case of hard English-type cookies), proceed as in (2).

(1) Add sufficient Na_3PO_4 soln (ca 5%) to bring mixt. to *p*H 7–8. Stir and break up material as much as possible. Cool to 40°, add 100 ml of pancreatin soln, **35.2(d)**. Stir thoroly and readjust to *p*H 7–8. Allow to stand 30 min. Stir and readjust *p*H.

(2) Estimate vol. of mixt. and adjust acidity to ca 1% by adding sufficient concd HCl. Boil until the mixt. becomes finely divided and so digested that it will not froth over when covered during boiling. Neutralize to ca *p*H 6 with NaOH soln. Add Na_3PO_4 soln to *p*H 8 and continue as in (1).

For white flour products, add 2 ml HCHO and digest overnight. For products made from whole wheat and rye flours and similar materials of high bran content, digest only 2–3 hrs.

Pour digested material thru a 5 or 8 inch No. 140 screen. While pouring, play a forcible stream of hot H_2O from the tap on this material. Wash well with a large stream of hot H_2O. After complete washing (no starchy material visible unattached to bran) wash twice alternately with alcohol and $CHCl_3$, in that order, and then give the material a thoro alcohol rinse and a final hot H_2O rinse.

Transfer material to filter paper if little residue remains or to a 1 or 2 l Wildman trap flask if a larger amount remains. Transfer bulk of material with a spoon. Rinse residue from screen with 60% alcohol from a wash bottle. Wash screen with forcible stream of hot H_2O, collecting final residue at one edge of screen and transferring to trap flask with stream of alcohol as above. Add 400 or 900 ml of 60% alcohol depending on size of trap flask.

Boil 20 min. Cool below 20° and add 20 or 40 ml of gasoline, fill flask with 60% alcohol and trap off in usual manner. Trap off a second time. Use care in stirring and during the addn of alcohol to prevent formation of an emulsion or the inclusion of air. If residue in flask tends to rise, stir material down 2 or 3 times. Filter trapped-off material and examine.

(6) The title of methods **35.32–35.33** (p. 715) was changed to "Whole and Degerminated Corn Meal, Corn Grits, Rye Meal, Wheat Meal, Whole Wheat Flour, Farina, and Semolina."

(7) Methods **35.29(b)**, **35.33**, **35.34**, **35.35**, and **35.36(b)**, **(c)**, and **(d)** were deleted and the following method for insects, insect parts, and rodent hairs was adopted first action as **35.33**:

Draw air thru the material in Büchner funnel, **35.32**, until the liquid has evapd. Air dry overnight or dry in drying oven at ca 80°C. for 1 hr. (*Caution:* In oven drying, phosgene is liberated and adequate ventilation must be provided.) Transfer residue to a 1 Wildman trap flask. Add 100 ml of 60% isopropyl alcohol previously satd with gasoline and mix thoroly. Wash down sides of flask with the alcohol-gasoline soln until ca 300 ml are added and soak 30 min. Trap off twice using 20–30 ml gasoline for each trapping and the 60% isopropyl alcohol satd with gasoline as

the flotation medium. In the first trapping, allow to stand 5 min. after stirring in the gasoline before filling flask. Filter and examine both trappings microscopically. Identify type of insect which contributed the fragments insofar as possible.

(8) The first action method **35.36(a)** (p. 716) was revised to read as follows:

Cream corn meal (corn flour).—Proceed as directed in **35.29(a)**.

(9) The title of the first action method "Identification of Tomato Rot Fragments," *This Journal*, **35**, 96 (1952), was changed to "Rot Fragments in Comminuted Tomato Products."

(10) The first action method for light and heavy filth in ground capsicums, **35.87** (p. 731) and the revised method **35.87**, *This Journal*, **36**, 90 (1953), were deleted and replaced by the following method which was adopted as first action:

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

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

Weigh 50 g pepper into 600 ml beaker and add 400 ml of petr. ether. Boil gently 30 min. on elec. hot plate adding petr. ether occasionally to keep vol. constant. Decant petr. ether onto smooth 15 cm filter papers in Büchner funnel. Add 400 ml CCl_4 and allow to stand 30 min., with occasional stirring. Decant pepper and solvent onto same 15 cm filter paper in Büchner funnel, leaving any heavy residue of sand and soil in beaker. Repeat decanting with CCl_4 if necessary to secure practically complete separation of spice materials from any heavy residue. Transfer residue from beaker to ashless filter and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite and determine sand and soil. Wash spice material in Büchner funnel with $CHCl_3$ and dry overnight or in an oven at 80°C.

Transfer dry residue to 600 ml beaker. Add 300–400 ml of H_2O, stirring until smooth. Add the filtered aq. ext. from 5 g pancreatin and mix. Adjust to *p*H 8 with Na_3PO_4 after ca 15 min., and again after ca 45 min. Add 5 drops of formaldehyde and digest overnight at 37–40°C. Transfer digested material to a 2 liter trap flask. Cautiously boil ca 10 min. until foaming partially subsides and cool to 20°C. Add H_2O to make ca 800 ml vol. and trap off twice with 25 and 15 ml gasoline, resp., in the usual manner. Combine trappings in beaker. Transfer contents of beaker to trap flask and fill with H_2O. Stir, and after 30 min. trap off into beaker and filter. Examine microscopically.

36. MICROBIOLOGICAL METHODS

(1) The following first action methods were deleted:

(a) Detecting and estimating numbers of thermophilic bacteria in sugar, **36.12–36.18** (p. 739).

(b) Examination of canned vegetable, **36.19–36.21** (p. 741).

37. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

38. RADIOACTIVITY

No additions, deletions, or other changes.

39. STANDARD SOLUTIONS

(1) The first action method for the standardization of hydrochloric acid by the constant boiling method, *This Journal*, **36**, 96 (1953) was revised as follows and adopted as first action:

Change the first four lines of the second paragraph (including the formula) to read:

Calc. air wt in grams (G) of this constant boiling HCl required to give one equivalent wt of HCl from one of the following equations:

For $P_o = 540–669$ mm Hg: $G = 162.255 + 0.02415\ P_o$

For $P_o = 670–780$ mm Hg: $G = 164.673 + 0.02039\ P_o$

40. NUTRITIONAL ADJUNCTS (VITAMINS)

(1) The following rapid method for thiamine in enriched flour was adopted as first action:

REAGENTS

(a) *Thymol blue pH indicator.*—Triturate 0.1 g of indicator in mortar with 4.3 ml of 0.05 N NaOH and dil. to vol. of 200 ml with H_2O.

(b) *Sodium chloride.*—Reagent grade.

(c) *Isobutyl alcohol.*—B.p. 106°–108°C. Isobutyl alcohol may be recovered as follows: Sep. the alcohol from the alk. ferricyanide, wash with ½ vol. of ca 0.1 N H_2SO_4 and distill in all glass equipment. Reserve first portion of distillate (constant boiling mixt. of alcohol and H_2O at ca 90°C.) for subsequent recovery. The distillate recovered at 106–108°C. should have reading not exceeding 3% of total photofluorometer scale.

(d) *Sulphuric acid.*—Ca N. 57 ml concd H_2SO_4 per 2000 ml H_2O.

(e) *Sulphuric acid.*—Ca 0.1 N. 5.7 ml concd H_2SO_4 per 2000 ml H_2O.

(f) *Sodium hydroxide soln.*—15% W/V.

(g) *Potassium ferricyanide soln, alkaline.*—Dissolve 40–50 mg $K_3Fe(CN)_6$ in 100 ml of the 15% NaOH. Use soln within 4 hrs.

(h) *Quinine sulfate stock soln and quinine sulfate standard soln.*—See **40.18 (m), (n)**.

(i) *Thiamin hydrochloride stock soln.*—Weigh accurately 22–25 mg of U.S.P. Thiamin Hydrochloride Reference Standard which has been kept in a desiccator over P_2O_5 at least 24 hrs. Keep stoppered during the weighing to avoid absorption of moisture. Dissolve in 20% alcohol adjusted to pH 3.5–4.3 with HCl and make to 1 liter with the acidified alcohol. Add sufficient addnl acidified alcohol to bring the concn to exactly 20 mmg per ml. Store in a cool place in a glass-stoppered light resistant bottle.

(j) *Thiamin hydrochloride standard solns.*—(1) From a portion of the stock soln that has been warmed to the proper temp., pipet 10 ml into 200 ml volumetric flask and dil. to vol. with the 0.1 N H_2SO_4 (1 ml = 1 mmg). Make an addnl diln by pipetting 40 ml of the intermediate standard into 200 ml volumetric flask and dil. to vol. with the 0.1 N H_2SO_4 (1 ml = 0.2 mmg) (Direct standard soln).

(2) Add NaCl to the standards in amount to give a final concn of ca 5% W/V if NaCl was added to the sample. (See note under "*Extraction.*")

EXTRACTION

Weigh sufficient sample to give a final sample soln with a thiamin content of ca 0.2 mmg/ml (*i.e.*, 4.54 g of flour for 100 ml or 9.07 for 200 ml of final vol.) and proceed by one of the following methods:

(a) (95–100°C. *digestion*).—Transfer weighed sample to a digestion bottle,* and add a vol. of ca 0.1 *N* H_2SO_4 that is ca 15 to 1 relating ml acid to g sample. The acid is added in 2 portions with vigorous stirring, using part of the acid to wash down sides of bottle. Place bottles in H_2O bath previously brought to 95–100°C. Stir at frequent intervals to keep solids in suspension during thickening stage (5–8 min.) and occasionally during the balance of the total heating time of 30 min. After hydrolysis has proceeded for ca 10 min., place a drop of the soln on spot plate and test with thymol blue. Soln should be distinctly red (*p*H 1–1.2). If not sufficiently acid (indicating presence of basic substances in sample) add ca *N* H_2SO_4 in 1.0 ml amounts until desired acidity is reached. Note amount of *N* acid required to supplement the 0.1 *N* acid and *repeat digestion* with new sample wt and the necessary mixture of *N* and 0.1 *N* acids. Cool to room temp. and make to required vol. with 0.1 *N* H_2SO_4.

Centrifuge mixt. until the supernatant liquid is clear or practically so, or filter thru paper. (Check filter paper for thiamin adsorption by comparing filtered and non-filtered thiamin standard soln.) Filtration may also be accomplished with suction employing sintered glass funnels and analytical filter aid. Discard first part of filtrate in amount of ca 0.1 total vol.

(b) *Autoclaved digestion.*—Follow directions of method **(a)** without addn of NaCl, except to autoclave at 5 lb pressure (108–109°C.) for 20 min. with a total heating time of not more than 35 min. including 5–10 min. to attain the desired pressure and ca 5 min. to reduce pressure. (It may be necessary to preheat autoclave to ca 100°C. previous to introduction of samples. *Release pressure gradually to avoid foaming and resultant loss of soln.* Erlenmeyer flasks of ca 800 ml capacity are recommended.)

OXIDATION

Transfer 5 ml aliquots of prepd sample soln and of standard thiamin soln to glass-stoppered centrifuge tubes or glass-stoppered bottles of about 35 ml capacity contg ca 1.5 g of NaCl. (Where excessive amounts of thiamin are found, make sufficient diln with 0.1 *N* H_2SO_4 so that 5 ml will contain ca 1.0 mmg. Swirl tubes or bottles gently to dissolve part of the salt. (*The precision and accuracy of results depends upon a uniform technique in carrying out the oxidation.*) For addn of oxidizing agent, use pipet which will deliver 3 ml in 1 sec. Place tip of pipet holding the alk. ferricyanide soln in neck of tube and hold it so that stream of soln will not hit side of tube. Give tube a gentle swirl to impart rotational motion in liquid and immediately add 3.0 ml of alk. ferricyanide. Remove pipet and swirl tube once again to insure adequate mixing. *Immediately thereafter* add 13 ml of isobutyl alcohol, stopper, and shake tube for 10–15 sec. After alcohol has been added to all tubes, they should be given an addnl shaking for ca 2 min. (The tubes may be combined in a shaker box for this addnl shaking.) Centrifuge for 2–3 min. Pipet ca 10 ml of the alcohol layer into photofluorometer cuvettes, or, if small bottles have been employed, the alcohol can be poured off. The shoulder of bottle will aid in sepn of the two liquids.

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* The addn of NaCl in amount to give a final concn of ca 5% w/v will aid in the subsequent sepn of the sample soln. The flour and salt should be thoroly mixed with a stirring rod previous to the addn of the 0.1 *N* H_2SO_4.

BLANKS

Run blanks on 5 ml of standard and assay solns, adding 3 ml of 15% NaOH in place of the alk. ferricyanide soln and employing the same technique described above. (Avoid contaminating blanks with minute amounts of alk. ferricyanide soln that may be on the tip of the isobutyl alcohol buret or pipet.)

THIOCHROME FLUORESCENCE MEASUREMENT

Measure fluorescence with a photofluorometer fitted with an input filter with peak transmittance at 365 mμ and an output filter effective in screening out the incident light. The recommended range for output filter is 400–475 mμ with peak transmittance close to 435 mμ. Make a standard curve by plotting mmg thiamin against readings corrected for blanks. By means of this curve, convert sample readings corrected for sample blanks to mmg thiamin and calc. to mg per pound. Suggested amounts of final standard soln are 1, 3, and 5 ml representing 0.2, 0.6, and 1.0 mmg thiamin, diluting all tubes to 5 ml with 0.1 *N* H_2SO_4. Use the quinine sulfate standard soln to govern reproducibility of the instrument.

(2) The following statement was added as a footnote to the official chemical method for nicotinic acid, *This Journal*, **34**, 99 (1951), **35**, 102 (1952), **36**, 96 (1953), after the heading "*Method for enriched food products and feeds:*"

For the extn of materials contg bran, after autoclaving with 1 *N* H_2SO_4 adjust the mixt. to ca *p*H 13 by addn of 10 *N* NaOH and allow to stand at room temp. for 15 min. Then adjust the *p*H to ca *p*H 4.5 by addn of (3+1) HCl and proceed as usual. For the extn of glutenous material with ca 1 ounce samples, use 400 ml 1 *N* H_2SO_4.

(3) The method for the assay of vitamin B_{12} feed supplements containing from approximately 1.0 milligrams to 10 milligrams vitamin B_{12} per pound, adopted first action, *This Journal*, **36**, 96 (1953), was replaced by the following method for the assay of vitamin B_{12} in materials containing approximately 0.1 micrograms or more of vitamin B_{12} per gram or per ml (0.05 milligrams per pound or more) and adopted as first action:

REAGENTS

(a) *Standard cyanocobalamin stock soln.*—To a suitable quantity of U.S.P. cyanocobalamin reference standard, accurately weighed, add sufficient 25% alcohol to make a soln, each ml to contain 1.0 mmg of cyanocobalamin. Store in a cool place and use no longer than 60 days.

(b) *Standard cyanocobalamin soln.*—To 10 ml of standard cyanocobalamin, stock soln (a) add 25 ml 1.0% $NaHSO_3$ in *p*H 4.5 phosphate-citrate buffer. Autoclave for 15 min. (121–123°C.) and dil. with H_2O to 500 ml. Dil. an aliquot of this soln 1000 times. Each ml represents 0.02 millimicrogram of cyanocobalamin. Prep. a fresh standard soln for each assay.

(c) *Basal medium stock soln.*—To prevent the possible formation of colloidal suspensions or ppts which may result in either slow growth or high blanks (or both), the following sequence of ingredient addns should be followed:

100 mg l-cystine and 100 mg d,l-tryptophane dissolved in ca 10 ml 1 *N* HCl.	
Adenine-guanine-uracil soln, (f)	5 ml
Xanthine soln, (g)	5 ml

Vitamin soln I, (h)	10 ml
Vitamin soln II, (i)	10 ml
Salt soln A, (j)	5 ml
Salt soln B, (k)	5 ml
Asparagine soln, (e)	5 ml
Acid-hydrolyzed casein soln, (d)	25 ml
Dextrose, anhyd	10 g
5 g sodium acetate, anhyd. and 1 g ascorbic acid dissolved in 100 ml H_2O	
Polysorbate 80 soln, (l)	5 ml

Adjust to *p*H 6.0 with NaOH soln, and finally add H_2O to 250 ml.

(d) *Acid-hydrolyzed casein soln.**—Mix 100 g vitamin-free casein with 500 ml dil. HCl (1+1) and reflux the mixt. for 8–12 hrs. Remove the HCl by distn under reduced pressure to a thick paste. Redissolve paste in H_2O, adjust *p*H to 3.5 ($\pm$0.1) with NaOH soln and add H_2O to 1000 ml. Add 20 g activated charcoal, stir 1 hr, and filter. Repeat the treatment with activated charcoal. Store under toluene in refrigerator at a temp. not below 10°C. Filter soln if a ppt forms on storage.

(e) *Asparagine soln.*—Dissolve 2.0 g l-asparagine in H_2O to make 200 ml. Store under toluene in refrigerator.

(f) *Adenine-guanine-uracil soln.*—Dissolve 0.2 g each of adenine sulfate, guanine hydrochloride, and uracil with the aid of heat, in 10 ml of 20% HCl, cool, and add H_2O to make 200 ml. Store under toluene in refrigerator.

(g) *Xanthine soln.*—Suspend 0.2 g xanthine in 30–40 ml H_2O, heat to ca 70°, add 6.0 ml of NH_4OH (40 ml dild to 100 ml with H_2O), and stir until solid is dissolved. Cool, and add H_2O to 200 ml. Store under toluene in refrigerator.

(h) *Vitamin soln I, riboflavin-thiamine-biotin-nicotinic acid soln.*—Dissolve 10 mg of riboflavin, 10 mg of thiamine hydrochloride, 0.1 mg of biotin, and 20 mg nicotinic acid in 0.02 *N* acetic acid to make 400 ml. Store, protected from light, under toluene in refrigerator.

(i) *Vitamin soln II, p-aminobenzoic acid-calcium pantothenate-pyridoxine-pyridoxal-pyridoxamine-folic acid soln.*—Dissolve 20 mg of p-aminobenzoic acid, 10 mg of Ca pantothenate, 40 mg of pyridoxine hydrochloride, 40 mg of pyridoxal hydrochloride, 8 mg of pyridoxamine dihydrochloride, and 2 mg of folic acid in 25% neutralized alcohol to make 400 ml. Store, protected from light, in refrigerator.

(j) *Salt soln A.*—Dissolve 10 g KH_2PO_4 and 10 g K_2HPO_4 in H_2O to make 200 ml. Add 2 drops HCl and store under toluene.

(k) *Salt soln B.*—Dissolve 4.0 g $MgSO_4 \cdot 7H_2O$, 0.2 g NaCl, 0.2 g $FeSO_4 \cdot 7H_2O$, and 0.2 g $MnSO_4 \cdot H_2O$ in H_2O to make 200 ml. Add 2 drops HCl and store under toluene.

(l) *Polysorbate 80 soln.*—Dissolve 20 g of polysorbate 80 in sufficient alcohol to make 200 ml. Store in refrigerator.

(m) *Tomato juice preparation.*—Centrifuge commercially canned tomato juice so that most of pulp is removed. Suspend analytical filter-aid (about 5 g/l) in the supernatant liquid and filter, with aid of reduced pressure, thru a layer of analytical filter-aid. Repeat if necessary until a clear, straw-colored filtrate is obtained. Store under toluene in refrigerator.

(n) *Culture medium.*—Dissolve 0.75 g water-soluble yeast ext., 0.75 g peptone, 1 g anhyd. dextrose, and 0.2 g KH_2PO_4 in 60–70 ml H_2O. Add 10 ml tomato juice prepn (m), and 1 ml polysorbate 80 soln (l). Adjust to *p*H 6.8 with NaOH soln, and add H_2O to 100 ml. Place 10 ml portions in test tubes, and plug with cotton.

* Commercial sources have been found satisfactory. If such a source is used, the suitability of each batch obtained should be demonstrated.

Sterilize tubes and contents in autoclave for 15 min. at 121–123°C. (exhaust line temp.). Cool as rapidly as possible to avoid color formation from overheating.

(o) *Suspension medium.*—Dil. measured vol. of basal medium stock soln with equal vol. H_2O. Place 10 ml portions of the dild medium in test tubes. Sterilize, and cool as for culture medium (n).

(p) *Stock culture of Lactobacillus leichmannii.*—To 100 ml culture medium add 1.0–1.5 g agar, and heat with stirring on steam bath until agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug with cotton, sterilize for 15 min. in autoclave at 121°–123° (exhaust line temp.), and allow tubes to cool in upright position. Prep. stab cultures in 3 or more tubes, using pure cultures of *Lactobacillus leichmannii.*† (Before using a fresh culture in this assay, make at least 10 successive transfers of the culture in a two-week period.) Incubate 6–24 hrs at any selected temp. between 30° and 37° but held constant to within ±0.5°, and finally store in refrigerator.

Prepare fresh stab cultures thrice weekly and do not use them for preparing the inoculum if more than 4 days old.

The activity of the microorganism can be increased by daily, or twice-daily transfer of the stab culture, and may be considered satisfactory when definite turbidity in the liquid inoculum can be observed 2–4 hrs after inoculation. With a slow growing culture a suitable response curve is seldom obtained, and may give rise to erratic results.

(q) *Inoculum.*—Make transfer of cells from stock culture of *Lactobacillus leichmannii* to sterile tube contg 10 ml culture medium. Incubate for 6–24 hrs at any selected temp. between 30° and 37° but held constant to within ±0.5°. Under aseptic conditions, centrifuge culture, and decant supernatant liquid. Suspend cells from culture in 10 ml sterile suspension medium. Dil. an aliquot with sterile suspension medium to give a transmittance of about 85%–90% (Evelyn and Lumetron),* 70%–80% (Coleman), or 55%–70% (Beckman Model B) when read against the suspension medium set at 100. The cell suspension so obtained is the inoculum.

TEST SOLUTION OF THE MATERIAL TO BE ASSAYED

Autoclave ca 1 g or 1 ml of sample, accurately measured, for 15 min. (121°–123°C.) in 25 ml of 0.1 *M* phosphate-citrate buffer at *p*H 4.5 (12.9 g Na_2HPO_4 (anhyd.), 11.47 g citric acid (monohydrate), and H_2O to make a l) contg 1.0% Na metabisulfite (the Na metabisulfite must be added to buffer just prior to use). Allow any undissolved particles to settle, or centrifuge if necessary. Dil. an aliquot of the clear soln with *water* so that the final test soln contains a vitamin B_{12} activity equivalent to ca 0.02 millimicrogram of cyanocobalamin. Addn of excess bisulfite to the assay tubes must be avoided to prevent inhibition of the test organism. This will not occur if amount of bisulfite in the assay soln does not exceed 0.025 mg/ml or if the assay tube does not contain more than 0.125 mg.

DETERMINATION

Cleanse hard glass test tubes, ca 20×150 mm, and other necessary glassware meticulously by suitable means because of the high sensitivity of the test organism to minute amounts of vitamin B_{12} activity and to traces of many cleansing agents.

To triplicate test tubes, add 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml, respec-

† Pure cultures of *Lactobacillus leichmannii* may be obtained from the American Type Culture Collection, 2029 M Street, N.W., Washington, D. C. as No. 7830.

* Instruments vary in sensitivity and hence in % transmission. As a result, the assay range (from inoculated blank to highest level of standard) will vary accordingly. The Evelyn and Lumetron % transmission response is ca ½ to ⅔ that of the Coleman and Beckman Model B, respectively. Because of this, % transmittancies are expressed in terms of the instrument used.

tively, of the standard cyanocobalamin soln. To each tube add 5.0 ml basal medium stock soln and sufficient H_2O to make 10 ml. Provide 6 similar tubes contg no standard cyanocobalamin soln.

To similar test tubes add *in triplicate* respectively, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, and 5.0 ml of the test soln of material to be assayed. To each tube add 5.0 ml of basal medium stock soln and sufficient H_2O to make 10 ml.

Mix, cover the tubes suitably to prevent bacterial contamination, and sterilize tubes and contents in autoclave for 5 min. at 121–123° C. (exhaust line temp.) arranging to reach this temp. in not more than 10 min. Cool as rapidly as practicable to avoid color formation from overheating. Take precautions to maintain uniformity of sterilizing and cooling conditions thruout the assay. Too close packing of tubes in the autoclave, or overloading of it, may cause variation in heating rate.

Aseptically inoculate each tube (except 3 tubes contg no standard cyanocobalamin soln–uninoculated blank) with 1 drop of inoculum. Incubate for 16–24 hours at any temp. between 30° and 37°C. but held constant to within ±0.5°C. until maximum turbidity is obtained, as demonstrated by a lack of significant change during a 2 hr period in the tubes contg the highest level of standard cyanocobalamin soln (0.1 millimicrogram). Read turbidity of tubes in a suitable instrument at a specific wavelength of between 540 and 660 mμ. In taking instrument reading, thoroly mix contents of each tube and transfer to optical glassware. Agitate each tube or cuvette to obtain a uniform suspension. A few sec. after agitation, a steady state is reached in which the galvanometer needle remains constant for 30 sec. or more, allowing sufficient time for an instrument reading. Allow ca the same time interval to elapse prior to each reading. A little practice will establish the proper time interval.

With uninoculated blank in instrument, set meter to read 100% transmittancy and read transmittancy of the inoculated blank (the inoculated tubes to which no standard cyanocobalamin soln has been added). Disregard results of an assay if contamination with a foreign organism is evident, or if the inoculated blank tubes give a reading of less than 90% transmission (Evelyn and Lumetron), 80% (Coleman), or 65% (Beckman), thereby indicating interference due to vitamin B_{12} activity in the basal medium stock soln or inoculum. Then with the inoculated blank in the instrument, set meter to read 100% transmittancy. Read transmittancy of tubes of the standard and sample series. Disregard results of an assay if transmittancy of tubes contg the highest level of standard cyanocobalamin soln (0.1 millimicrogram) exceeds 65% (Evelyn and Lumetron), 45% (Coleman), or 30% (Beckman) of transmittancy readings of inoculated blank, e.g. setting the % transmittancy of inoculated blank at 100 (Beckman), then the 5 ml level of standard must have % transmittancy of 30 or less.

CALCULATION

Prep. a standard concn response curve by plotting % transmittancy readings for each level of the standard cyanocobalamin soln used, against millimicrograms of cyanocobalamin contained in the respective tubes. Draw the smooth curve which by visual inspection appears to fit best the plotted points. From this standard curve det. by interpolation for each tube the amount of cyanocobalamin equivalent to the vitamin B_{12} activity of each ml of the test soln of the material to be assayed.

Since in microbial assays occasional inexplicable aberrant values are obtained in individual tubes, inspect the series of values and set aside any which vary markedly from most of the series. Strike a provisional av. of the remaining values, and set aside any of the latter which are less than 90% and more than 110% of the provisional av. If less than 10 of the 15 original values remain, the data are insufficient for calcg the potency; if 10 or more values remain, calc. the potency from the

av. Disregard any assay in which the calcd potency is less than 75% or more than 125% of the assumed potency.

ERRATA AND EMENDATIONS, METHODS OF ANALYSIS, A.O.A.C., 1950

The errors that are reported from time to time by those using this book will be published in *This Journal*. The following changes should be made.

Section	*Page*	
16.33 last line, par. 2	284	Interchange figures "2.92" and "9.34."
17.7	291 line 11	Add after "ice–H_2O temp.)" the phrase "2 ml 12% $Na_2WO_4 \cdot 2H_2O$."
35.54	721 line 7	Change H_2O_0 to H_2O.

Note: The report on "Changes in Methods" should be consulted.

ERRATA FOR THIS JOURNAL

This Journal, **35**, 96 (1952) second and third lines of last paragraph	Change "gentian" to "crystal."
Ibid., **35**. 97, 98 (1952)	Remove "(before reduction)," in both cases, from the titles of Plates 1 and 2.
Ibid., **36**. 68 (1953)	Under (c), change "Lecithin" to "Letheen."
Ibid., **36**. 665 (1953)	Table 1: change sieve size for samples number 8–12, incl., from −30 to −150.
Ibid., **36**. 1081 (1953)	Table 4: for Sample No. 3, change figure 359 under the column "Official Method (Hillig)" to 259.
Ibid., **36**. 1119 (1953)	Line 4 of Paragraph 2: change "1-ethoxy-3-*p*-nitrophenylazo-5-propenylphenol" to "2-ethoxy-4-(*p*-nitrophenylazo)-5-propenylphenol." Propenylguaethol is manufactured under the registered trade name, Vanitrope ®, by the Fine Chemicals Division of Shulton, Inc., New York (not Shulton Brothers Co., as stated in **36**, 1121). The article by Mitchell, **36**, 1123, deals with the identical material.

REPORT OF THE SECRETARY-TREASURER

WILLIAM HORWITZ

The Executive Committee was called to order by President Fisher at 2:00 P.M. on Sunday, October 11, 1953, in the West Room of the Shoreham Hotel. All members were present. The audit of the accounts of the Association as reported by Bisselle, Underwood & Company was presented and accepted. The statement of the financial condition of the Association follows:

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, INC.

STATEMENT OF FINANCIAL CONDITION AS OF SEPTEMBER 30, 1953

ASSETS

Current Assets:	
Cash, Lincoln National Bank	$ 18,770.51
Cash on hand	180.65
Office cash fund	64.95
	$ 19,016.11
Accounts receivable	3,061.72
Accrued interest receivable	250.00
Inventories	13,137.10
Total Current Assets	$ 35,464.93
Fixed Assets:	
Furniture and fixtures	1,844.01
Investments	73,584.00
Total Assets	$110,892.94

LIABILITIES

Current Liabilities:	
Accrued and withheld payroll taxes	$235.50

SURPLUS

Balance, September 24, 1952	$106,731.74
Add net income for the fiscal year ended September 30, 1953	3,925.70
Balance, September 30, 1953	110,657.44
Total Liabilities and Surplus	$110,892.94

It was moved, seconded, and passed that the advertising rates for the *Journal* be increased.

It was moved, seconded, and passed that the Secretary enter into a cancelable agreement with J. S. Canner & Company, Inc., Boston, Mass. for publishing back volumes of *The Journal* on microcards.

It was moved, seconded, and passed that the President appoint a chairman of the Committee on Revision of *Official Methods of Analysis* and that the Secretary be instructed to incur all the necessary expenses incident to the publication of the Eighth Edition.

It was moved, seconded, and passed that the Association invite the Presidents of the Association of Official Agricultural Chemists, the Association of American Fertilizer Control Officials, the Association of American Feed Control Officials, and the Association of Economic Poisons Control Officials to its annual banquet and incur the necessary expenses.

Approved.

REPORT OF THE COMMITTEE ON NECROLOGY

Since the previous meeting of the Committee on Necrology in September of this year, there has come to our attention the passing of the following co-workers whose names we hold in reverence:

DR. CHARLES W. BACON

Dr. Charles W. Bacon, of the Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, died on March 19, 1953, at his home in Washington, D. C.

Dr. Bacon was born at Worcester, Massachusetts, in 1886; he received his B.S. degree in 1906, his M.A. in 1907, and his Ph.D. in 1911 from Clark University. In 1910 he joined the Bureau of Plant Industry of the Department of Agriculture as an associate physiologist. His lifework, spend in the Department of Agriculture, is noted for his studies on the physiology and biochemistry of the tobacco plant. He participated in fundamental investigations of the nutritional requirements of plants, and in studies of tobacco varieties and the curing of tobacco from a chemical point of view.

R. EARLE DICKEY

Mr. Dickey, State Chemist of Delaware, was stricken with coronary thrombosis on January 2, 1953, and his death occurred on January 24.

Mr. Dickey was born at Stanton, Delaware, in 1893; he attended Newark High School and obtained a degree in agriculture from the University of Delaware in 1919.

In April, 1923, Mr. Dickey was first employed by the State Board of Agriculture as an inspector and chemist, and at the time of his death he

had occupied the position of State Chemist for six years. In addition to almost thirty years of faithful service to Delaware farmers, he was also very active in religious, fraternal, and civic affairs.

ERNEST H. GRANT

Ernest H. Grant, who retired in 1950 after thirty-two years of Food and Drug work, died suddenly at his home in Melrose, Massachusetts, on March 15, 1953, his sixty-fifth birthday.

Mr. Grant received the degree of B.S. in Chemical Engineering from Ohio State University at Columbus in 1910, and from 1910 to 1919 he served with the Bureau of Chemistry, U. S. Department of Agriculture, as a chemist specializing in the analysis of drugs under the Food and Drug Act. In the course of nine years he was stationed at New Orleans, at Puerto Rico, and at New York.

In 1919 he resigned from the Department of Agriculture to enter the commercial field. In 1927, he returned to government service with the Food, Drug, and Insecticide Administration and was stationed at Baltimore. He later transferred to Boston, where he set up a specialized drug laboratory (before that time, drug samples had been sent to the drug laboratories at New York or Baltimore). Mr. Grant continued his drug work at Boston until his retirement. He also served the A.O.A.C. as Associate Referee and as Referee on drug analysis.

Mr. Grant's knowledge of drugs and drug analyses was the quiet kind that made even the most complicated analysis seem easy in his hands. On his visits to the laboratory after his retirement, it was not unusual to see two or three of the Boston District chemists "ganging up" on him for answers to questions which had perplexed them.

Entering the Food and Drug Service in 1910, Mr. Grant was another of the real old-timers, although he did not seem old to his colleagues. Quiet and always good-natured, he was ever willing to share his knowledge with his fellow workers, and nothing delighted him more than to help a fellow chemist.

GEORGE E. GRATTAN

Mr. Grattan was for many years Chief of the Plant Products Chemical and Micro-analytical Laboratories in the Department of Agriculture, Ottawa, Canada, prior to his service in the Department of National Defense. He was born at St. Catherines, Ontario, and received his early education there. He obtained the degree of Master of Science from McMaster University in 1915.

Mr. Grattan for many years was active in the Association of Official Agricultural Chemists, and served both as Associate Referee and as a

member of Committee A. His death occurred in an Ottawa hospital on April 30, 1953, in his sixty-third year.

Austin E. Lowe

The death of Austin E. Lowe, who retired in 1943 as Chief of New York Station, is recorded with deep regret. He succumbed to a heart attack on July 20 at his home in Alhambra, California, at seventy-one years of age.

Mr. Lowe entered the service in 1912 as a food and drug inspector. An experienced pharmacist at the time of his appointment, his work as an inspector attracted attention from the beginning. He served as Chief Inspector at Baltimore and Boston from 1924 to 1928, Assistant to the Chief of the Eastern District until 1933, and Chief of St. Louis and New York Stations for the last ten years of his Food and Drug work. He retired voluntarily in 1943, secure in the esteem and good will of all his associates.

A. V. H. Mory

A. V. H. Mory, a former member of the Bureau of Chemistry and formerly associate editor of the *Journal of Industrial and Engineering Chemistry*, died in Amherst, Massachusetts, at the age of 83.

Mr. Mory was appointed chemist in charge of the Kansas City Laboratory when the 1906 Food and Drug Act was passed. He left government service to work with Armour and Company, and later with Sears, Roebuck and Company, where he established and developed the merchandise laboratory and was responsible for the discovery and development of young men with administrative ability. One of Mr. Mory's proteges, Donald Nelson, later became director of the War Production Board.

Dr. Henry E. Moskey

The death of Henry E. Moskey, F. D. A. Veterinary Medical Director, on August 24, 1953, following a heart attack, is recorded with deep regret. He was buried at Arlington National Cemetery.

Dr. Moskey was born at Hyattsville, Maryland, in 1892; his government career began in 1911 when he became a messenger in the Bureau of Animal Industry. He was promoted in turn to skilled laborer and to clerk typist, and then took time out to serve in World War I and to complete work for a V. M. D. at the University of Pennsylvania. He returned to B. A. I. in 1920 as a veterinarian, and for seven years he specialized in bacteriology and the eradication of contagious diseases of animals in Texas, California, and other areas.

In 1927 he transferred to the Food and Drug Administration to work

on insecticides. The next year he took charge of Food and Drug Administration's veterinary work, a task that has grown in scope and complexity continuously throughout his service.

Food and Drug Commissioner C. W. Crawford, commenting on Dr. Moskey's work in exposing a host of worthless remedies for livestock and poultry and encouraging the adoption by the veterinary medical industry of new and effective chemotherapeutic agents, said: "The value of these contributions to the public welfare can scarcely be estimated."

The entire Food and Drug Administration feels a personal loss at the passing of a friend and respected associate, a man of good humor, professional ability, and integrity.

Dr. Mary E. Pennington

Dr. Mary E. Pennington, a member of the Bureau of Chemistry from 1905 until 1919, died in New York on December 27, 1952, at the age of 80. She was born at Nashville, Tennessee, in 1872.

Serving as a bacteriologist and chemist, in Philadelphia, she pioneered in the study of refrigeration and preservation of goods, and specialized in poultry and eggs.

After leaving the government, Dr. Pennington established offices in New York City and served as a consultant on refrigeration problems. In recognition of her work with the War Food Administration during World War I, she received a Notable Service Medal from Herbert Hoover, and in 1940 she was presented with the Garvan Gold Medal, the highest award to women given by the American Chemical Society.

O. Eugene Shostrom

Mr. O. Eugene Shostrom, chemist with the Northwest Branch of the National Canners Association, died on February 23, at the age of fifty-eight, after a short illness. He was well known to the entire salmon industry and to local, State, and Federal food law enforcement officials through his close association with the "Better Salmon Control Plan." One of his important contributions to the salmon canning industry is the method he devised for reconditioning canned salmon condemned under the control plan, which has resulted in the salvage of large amounts of good wholesome fish by the industry. Mr. Shostrom's contributions have been an important factor in the success of the control plan.

Dr. William W. Skinner

A separate biography of Dr. Skinner appeared in the August, 1953, issue of *This Journal.*

DANIEL M. WALSH

The death of Daniel M. Walsh, following a heart attack at his home in Rutland, Vermont, on December 25, 1952, is reported with regret. Mr. Walsh entered the service of the Department of Agriculture in June, 1907, with the first group of food and drug inspectors, was stationed in St. Paul, Pittsburgh, Buffalo, and Boston, and became chief of Baltimore Station in 1920. He was chief of New York Station from 1934 until 1938, when he retired because of ill health.

ELMER E. FLECK
ELMER R. TOBEY
WALLACE L. HALL, *Chairman*

REPORT OF THE COMMITTEE ON RESOLUTIONS

Whereas, The success of the Association during the past year is due in large part to the efforts of our officers who have faithfully carried out their duties and responsibilities and have thoughtfully planned and efficiently conducted this, the Sixty-seventh Annual Meeting of this organization: Now, Therefore,

Be It Resolved, That we express our earnest appreciation to President H. J. Fisher, Vice-President E. L. Griffin, and Secretary-Treasurer William Horwitz for their excellent and faithful service.

Whereas, The chief objective of this organization is to promote the development of reliable analytical methods and their acceptance for regulatory purposes and for general scientific use; and

Whereas, The attainment of these objectives is possible only through the exacting work of the Committee on Recommendations of Referees, the members of the several other committees, our Referees, Associate Referees, and collaborating analysts: Now, Therefore,

Be It Resolved, That we express our appreciation to these workers for having given of their time and energy to this work.

Whereas, The success of our work and the acceptance of our methods depend upon the active participation of workers in the many government agencies and commercial institutions in the United States and Canada: Now, Therefore,

Be It Resolved, That we thank those organizations which have made it possible for so many individuals to participate actively in research on the development of methods and to participate in this meeting.

Whereas, The results of our efforts are largely set forth in *Official Meth-*

ods of Analysis and our *Journal*, the preparation of which falls upon a small group of our members: Now, Therefore,

Be It Resolved, That we express to the members of the Editorial Board, and to those who assisted them, our appreciation of their efforts in accomplishing these difficult assignments.

Whereas, The success of this meeting has depended upon adequate assembly rooms and facilities: Now, Therefore,

Be It Resolved, That this Association request its Secretary to express to the management of the Shoreham Hotel the thanks of our Association for making rooms and other facilities available, and for the many courtesies shown our members during this annual meeting.

VIRDELL E. MUNSEY
RICHARD T. MERWIN
ALLEN B. LEMMON, *Chairman*

Approved.

REPORT OF THE COMMITTEE ON NOMINATIONS

Your Committee proposes the following nominees and moves their election to the respective offices, as designated:

President, E. L. Griffin, Production and Marketing Administration, U. S. Department of Agriculture, Washington, D. C.

Vice-President, W. F. Reindollar, Department of Health, Baltimore, Md.

Secretary-Treasurer, William Horwitz, Food and Drug Administration, Department of Health, Education and Welfare, Washington, D. C.

Secretary-Treasurer Emeritus and Ex Officio Member of the Executive Committee, Henry A. Lepper (in succession to our lamented W. W. Skinner), Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C.

As additional members of the Executive Committee: K. D. Jacob, Beltsville, Md.; M. P. Etheredge, State College, Miss.; Frank Vorhes, Jr., Washington, D. C.; and H. J. Fisher, New Haven, Conn.

E. A. EPPS
J. J. T. GRAHAM
W. H. MACINTIRE, *Chairman*

Approved.

CONTRIBUTED PAPERS

SYMPOSIUM ON EXTRANEOUS MATERIALS IN FOODS AND DRUGS*

INTRODUCTION

By KENTON L. HARRIS
(Division of Microbiology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.

It is quite apparent from this year's program that the portion devoted to Extraneous Materials in Food and Drug Products is organized in a manner very different from that of previous years. A (sometimes dreary) recitation of methods detail has been summarized and tucked away for future study and reference, and we are eager to devote the remainder of our time to a series of papers that should be of interest to both the plant manager and the laboratory chemist. So far as management is concerned we hope that these papers will give some insight into the problems of microanalytical procedures. To the laboratory chemist, we present these papers as reports on current problems and technics that will help us evaluate our present position and possibly point the way toward promising avenues of future investigation.

. . . . . .

Although many of you are chemists, the problems encountered in this field of investigation are often biological. Moreover, in this relatively new field, it wasn't until the Sixth Edition of *Official Methods of Analysis of the A.O.A.C.* appeared that recognition was given to methods for the detection of rodent and insect filth and to many of the mold count procedures. In this work, then, we surely have not reached a static position in respect to methodology, and we welcome suggestions that lead to new avenues of approach. As our methods have gained more widespread use, other organizations have worked with them and have studied some of them collaboratively. Frankly, some of the results were not too encouraging. It was finally realized that differences were due, at least in part, to the fact that methods were being used in a manner which reflected individual laboratory irregularities. Moreover, the counts were made by analysts possessing varying degrees of skill in recognizing insect and rodent debris.

Results by some of the methods may be difficult to interpret; with others (as in any analytical work where there is a sampling problem for

* Presented at the Sixty-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 12, 13, and 14, 1953, at Washington, D. C.

insoluble constituents) there are problems in obtaining comparable results from aliquot to aliquot. Repeated discussions, especially in the cereal field, have indicated that one of the largest sources of variation lies with the analysts who are highly skilled in other fields but who have a microanalytical problem thrust upon them before they have been adequately trained. Some professional organizations have instigated training programs and check microanalytical services in this field. Another organization is compiling a laboratory manual. We look forward to continued improvement in our methods and in their interpretation.

RECENT ADVANCES IN EXTRACTION TECHNIC*

By MARYVEE G. YAKOWITZ

(Division of Microbiology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, 25, D.C.)

A. SURFACE ACTIVE AND COMPLEXING AGENTS

As stated in Food and Drug Circular No. 1,[1] "The prime objective in making the Wildman trap extraction is to float out the insect and rodent filth while keeping down most of the food material." In many cases, this objective is difficult to achieve, for many foods have individual characteristics which necessitate modification of the basic trapping method.

Two years ago, a method was introduced, for determining light filth in Spanish peanuts, which made use of the surface-active agent, Tween 80. The addition of this agent to the 60 per cent alcohol flotation medium was found to be useful in preventing peanut skin hulls from rising with the gasoline layer. Formerly, these floating hulls had impeded the recovery of filth elements, and as a result, many filter papers required examination. Although the reagent showed promise when applied to extraction technics for certain other products, its use was not extensive. In some instances, older methods were preferred in which established principles and technics were applied, and which accordingly provided a uniform basis for appraising results.

For a number of other products, however, existing gasoline extraction procedures were not accomplishing the necessary separation of filth from the mass of food material. Notable among these were such items as finely granulated black walnuts, sage and certain other spices, rye flour, and bran. It now appears that the combined use of the surface-active agent,

* Presented at the Sixty-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 12, 13, and 14, 1953, at Washington, D. C.

[1] *Microanalysis of Food and Drug Products*, Food and Drug Circular No. 1, p. 21 (1944). (For sale by the Supt. of Documents, U. S. Government Printing Office, Washington, D. C.).

Tween 80, and a chelating agent, Versene,[2] in gasoline–60 per cent alcohol flotation procedures may remove these various foodstuffs from our list of flotation trouble-makers. The details of the procedure are given in a proposed method for granulated nutmeats reported by the Associate Referee on Extraneous Materials in Nut Products.[3]

Evidently, phenomena involving differential wetting, alterations in surface tension, and solubility come into play when these agents are used. Certain precautions appear to be necessary. The Versene reagent is hygroscopic and should be weighed and added quickly so as to minimize its exposure to air. Time also seems to be an important factor in dealing with these surface-active and complexing agents during their addition to the extraction flask. The more rapidly these reagents are added in the prescribed manner to the trap flask, the gasoline mixture is made, and the flask is filled with 60 per cent alcohol, the more successful appears to be the recovery of filth elements. Stirring during the first fifteen minutes of standing should be infrequent so as to permit the gasoline to separate and collect. Once the gasoline interface layer is formed, it should not be broken by stirring it back down into the 60 per cent alcohol mixture. (This habit of working with the sample in the flask during the standing period will be difficult for most analysts to overcome.) Finally, due caution should be taken not to disturb the interface layer when the plunger is raised for the trapping operation.

There are a number of other substances which may yield a better filth recovery (with cleaner separation) when subjected to the Tween 80–Versene flotation technique. Ground sage responds nicely to the new separation procedure if it is first boiled in the specified quantity of 60 per cent alcohol (to expel air from sage hairs) and cooled. It should also result in the more expeditious handling of a number of other materials such as crude drugs and herb mixtures. Mr. J. E. Roe of the Denver District of the Food and Drug Administration obtained better recoveries of insect fragments when he compared the Tween 80–Versene flotation procedure and the present A.O.A.C. method with duplicate 50 gram samples of corn meal.

The complex properties of these new reagents make it necessary to resort to trial and error laboratory work rather than to a theoretical approach in order to learn their value in filth procedures.

B. ENZYMIC DIGESTION

The present A.O.A.C. method **35.28 (b)** for determining total filth in bakery products and alimentary pastes includes boiling the sample with

[2] Tetra sodium salt of ethylene diamine tetra acetic acid. Versene, Regular, Powder—Bersworth Chemical Co., Framingham, Mass.
Subsequently, Sequestrene Na$_4$, Alrose Chemical Co., Box 1294, Providence 1, R. I., was found to be satisfactory.
[3] This report will appear in the August 15, 1954, Number of *This Journal*.

dilute acid, neutralizing, digesting with pancreatin, and extracting the resulting mixture with gasoline to trap off the filth particles. Frequently, the nature of the boiled and digested mixture renders it impossible to obtain a satisfactory gasoline extract because of troublesome emulsions. A method for these types of foodstuffs (essentially an adaptation of the A.O.A.C. method **35.29 (a)** for the pancreatin digestion of flour has been evolved. The new method for direct trapping of baked products will be described in detail in the report of the Associate Referee for Extraneous Materials in Baked Products and Cereals.[4] In brief, this method stresses the importance of proper preparation of the material for the digestion procedure and avoids the use of the boiling procedure which, incidentally, seems to have been the cause of much difficulty in the present A.O.A.C. direct extraction procedure.

It was also found that the method of digestion described above seems to be useful in the preparation of cheese samples for filtration through paper.

It was thought that some of the newer enzymes might be superior to pancreatin for the digestion step. Experiments involving Rhozyme R–48, Rhozyme P–11, Ficin, Pectinol, Seachem, and a new proteinase gave unsatisfactory recoveries compared with pancreatin. A gelatinous emulsion usually formed with the gasoline layer and although it apparently separated clearly and cleanly, the emulsions may have accounted for low recoveries with bakery products and alimentary pastes. No attempt has yet been made to explore the possibilities of combinations or mixtures of more recently developed enzymes.

Pancreatin digestion has long been a valuable tool for releasing filth elements from various foods. More successful digesting reagents may soon be found. In any event, adaptations of enzymic technics should not be overlooked in attempts to solve many of our filth separation problems.

SOME COLLABORATIVE INVESTIGATIONS OF METHODS FOR ISOLATING EXTRANEOUS MATERIAL FROM WHITE FLOUR*

By E. J. KITELEY (International Milling Co., Minneapolis, Minn.), Chairman, A.A.C.C. Sanitation Methods Committee

The primary function of the Methods Committee of the American Association of Cereal Chemists is to investigate, evaluate, improve, and

[4] This report will appear in the August 15, 1954, Number of *This Journal*.

* Presented at the Sixty-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 12, 13, and 14, 1953, at Washington, D. C.

standardize methods used in cereal chemistry for assessing the quality of cereal grains and their products. While the scope of their work does not include the invention of new methods, it is inevitable that during their activities, new methods may be evolved. Thus, their objectives are similar to those of the A.O.A.C.

The first A.A.C.C. Sanitation Methods Committee was formed in November, 1945, with Niles Walker as chairman. The committee report was published in *Cereal Chemistry*, **24**, 39 (1947). The report described three developments in technic: (*a*) The use of a one liter Squibb separatory funnel in place of the Wildman trap flask; (*b*) The use of dilute HCl for digesting the flour; and (*c*) Mixing of oil and extraneous particles by boiling the mixture for ten to twenty minutes.

The results obtained by the collaborators varied widely. In one sample of flour, the reported range was from 32 to 855 microscopic insect fragments per pound. Subsequent A.A.C.C. collaborators showed no better agreement (1, 2). Koehn (2) observed that "agreement among laboratories is still far from perfect," and "it seems apparent that much of the difficulty in obtaining consistent numerical results lies in the appraisal made by each observer as to what does and does not constitute an insect fragment."

This lack of agreement seriously handicapped efforts to evaluate technics for isolating extraneous matter in cereal products. In an attempt to obtain better agreement among mill technicians, the Millers' National Federation in 1951 appointed a committee to study the problem. This committee organized six fragment counting schools in the principal flour milling areas of the United States, and mill technicians and others who routinely made tests for extraneous matter in flour were invited to attend. A pamphlet, entitled *Training Material for Insect Fragment Counting Conferences*, was prepared and supplied to participants in these schools. It states in part: "Recent well organized, collaborative studies demonstrated that while the total number of insect fragments reported by individuals on any given slide may vary considerably, these experts agree perfectly when examining any one fragment. It is therefore concluded that a very important cause for lack of agreement in fragment counting is the difference in individual visual acuity."

INVESTIGATION OF DIFFERENCES IN FRAGMENT COUNTS AMONG ANALYSTS

In order to determine the causes of the variation in gross counts among technicians, Cory (3) prepared and distributed two paraffin-coated, cross-lined, numbered filter papers containing a quantity of typical insect and rodent hair fragments removed from flour. Twelve collaborators examined both filter papers and reported the total number and

location of identified fragments. The visual acuity rating of each collaborator was established in two ways:

(*a*) "Confirmed" fragments were established when five or more collaborators reported them. Each collaborator was rated by the percentage of confirmed fragments he reported.

(*b*) The number of fragments with indisputable diagnostic characteristics (mandibles, legs, antennae fragments, etc.) were recorded for each area on the filter paper. The percentage of these fragments identified by each collaborator was determined.

Ratings for the twelve collaborators by Method (*a*) ranged from about 55 to 85 per cent and by Method (*b*) from 31 to 90 per cent. The studies also showed that, while different analysts might report the same number of fragments from the same filter paper, they were not counting the same particles. For instance, three collaborators reported a total of 82 frag-

TABLE 1.—*Effect of speed of counting*

CHARGE NO.	ANALYST NO. 1			ANALYST NO. 2		
	5 MIN.	15 MIN.	25 MIN.	5 MIN.	15 MIN.	25 MIN.
	(*Insect Fragments*)			(*Insect Fragments*)		
Flour Sample No. 1						
1	47	61	65	57	72	71
2	46	57	63	60	75	81
3	39	56	57	55	74	72
4	47	50	58	70	78	78
5	46	59	69	57	64	69
6	59	72	88	63	81	82
7	41	64	62	85	85	91
8	53	69	71	71	86	83
Av.	47.2	61.0	66.6	64.8	76.9	78.4
Per cent low	29.1	8.4		17.3	1.9	
Flour Sample No. 2						
1	105	149	150	160	172	173
2	95	140	145	161	169	186
3	121	190	200	151	173	183
4	103	155	170	163	190	199
5	122	180	188	157	190	191
6	114	150	175	121	160	159
7	101	148	154	139	171	173
8	115	167	195	150	190	201
Av.	109.6	159.9	172.1	150.3	176.9	183.1
Per cent low	36.3	7.1		17.9	3.4	

TABLE 2.—*Effect of fatigue on analyst's ability to identify fragments*
(Flour Sample No. 2)

CHARGE	ANALYST NO. 1		ANALYST NO. 2	
	MORNING COUNT	AFTERNOON COUNT	MORNING COUNT	AFTERNOON COUNT
1	173	170	187	187
2	186	165	172	170
3	191	179	194	181
4	161	163	200	199
5	165	141	167	170
6	172	156	167	160
7	169	150	188	176
8	170	145	172	180
Av.	173.4	158.6	180.9	177.9
Per cent low		8.5		1.7

ments on one filter paper. However, only 43 fragments on the filter paper were reported by all three.

This work is being expanded during the coming year in order to: (*a*) improve the accuracy and skill of collaborators in identifying extraneous matter; and as a result, (*b*) narrow down the wide differences between analysts, and, if necessary, rule out those whose accuracy is not good enough for collaborative studies.

In order to study further the cause of differences in counts, two analysts studied the effect of: (*a*) speed of scanning the filter paper; and (*b*) effect of fatigue.

Eight replicate filter papers were prepared from each of two samples of flour. Each analyst examined and counted the insect fragments on the papers in approximately five, fifteen, and twenty-five minutes, in that order. The results are recorded in Table 1.

In order to check the effect of fatigue on the counting ability of the analyst, eight replicate filter papers prepared from flour sample No. 2 were counted at the beginning of a working day and again at the end of the day. Between the morning and afternoon counts, each analyst had examined 8 to 15 filter papers not involved in this study. An attempt was made to take the same care and time for each counting. The results are listed in Table 2.

The figures in Tables 1 and 2 indicate that analyst No. 2 can count the filter papers with greater speed and is less affected by fatigue. Analyst No. 2 is about twenty-five years younger than Analyst No. 1. Both work in the same laboratory, and the differences indicated in these figures were also observed in their routine work. In routine work, the speed of counting filter papers is related to the number of insect and rodent hair fragments and the amount of plant tissues on the paper.

STUDY OF METHODS OF ISOLATING EXTRANEOUS MATTER IN FLOUR

In June, 1952, a subcommittee was formed to study the difference, if any, in recoveries by various methods in common use. The subcommittee was charged with investigating: (*a*) methods used by laboratories; (*b*) comparison of the HCl digestion-mineral oil method and the HCl digestion-gasoline method; (*c*) comparison of the Wildman trap flask and Squibb separatory funnel; and (*d*) comparison of recoveries from first and second trappings. To evaluate items *b*, *c*, and *d*, ten collaborators were supplied with four samples of flour, each sample to be tested in duplicate. Taken in order, the conclusions of this survey and study were:

(1) One hundred per cent of the analysts reported that they used HCl for digesting white flour. A few analysts used pancreatin digestion for bread and whole wheat flour.

Eighty-seven per cent used mineral oil and 13 per cent used gasoline. Seventy-nine per cent used the separatory funnel and 21 per cent used the Wildman trap flask.

Ninety-one per cent used a 50 gram charge, 8 per cent used 113 grams, and 1 per cent used 100 grams.

About 53 per cent used either methyl green or methylene blue as a selective stain of recovered particles, and 47 per cent used no stain.

(2) The recovery of fragments by HCl-mineral oil and HCl-gasoline methods was not significantly different. However, the difference in counts among laboratories was again extremely wide; in one flour, a range in insect fragments of 11 to 348 was reported for a 50 gram charge.

(3) The collaborators' counts averaged 65 per cent higher for the separatory funnel than for the Wildman trap flask.

(4) An average of 15 per cent of the total insect fragments was recovered in the second trapping.

One analyst with considerable experience in the technics for isolating and counting extraneous matter made a somewhat more detailed study of the methods. In this experiment, two samples of commercially milled flours were used and 8 replicate tests were made in comparing these commonly used methods:

(1) Pancreatin digestion–gasoline flotation method (4).
(2) HCl digestion–gasoline flotation method (4).
(3) HCl digestion–mineral oil flotation method (5).

In the first two methods, the oil and the extraneous particles are mixed manually, and in the last method the mixing is accomplished by the convection currents during boiling in the presence of the mineral oil. In Table 3 are shown the results of this comparison.

TABLE 3.—*Comparison of results with Methods 1, 2, and 3*

SAMPLE 1		SAMPLE 2	
INSECT FRAGMENTS	RODENT HAIRS	INSECT FRAGMENTS	RODENT HAIRS
Method 1: F.D.A. pancreatin–gasoline–Wildman flask			
65	0	150	1
63	1	145	0
57	0	200	0
58	1	170	1
69	2	188	0
88	4	175	2
62	2	154	0
71	1	195	1
Av. 66.6	1.4	172.1	0.63
Method 2: F.D.A. HCl–gasoline–Wildman trap flask			
41	1	92	1
52	0	126	1
48	0	117	1
65	1	115	1
58	3	126	4
63	0	131	1
64	2	130	4
48	1	129	2
Av. 54.9	1.0	120.8	1.9
Method 3: A.A.C.C. HCl–mineral oil-gasoline–separatory funnel			
60	1	140	2
58	1	138	1
57	2	131	4
66	1	127	1
56	2	145	2
76	6	142	0
60	1	146	0
61	2	138	1
Av. 61.8	2.0	138.4	1.4

Recoveries with the Wildman trap flasks were then compared with those obtained with the separatory funnel. The results are given in Table 4. In the third experiment (Table 5) the recovery of extraneous matter with one and with two trappings is shown.

TABLE 4.—*Comparison of two liter Wildman trap flask with one liter separatory funnel*

WILDMAN TRAP FLASK				SEPARATORY FUNNEL			
SAMPLE 1		SAMPLE 2		SAMPLE 1		SAMPLE 2	
I.F.	R.H.	I.F.	R.H.	I.F.	R.H.	I.F.	R.H.
Method 1: F.D.A. pancreatin–gasoline							
65	0	150	1	81	2	170	0
63	1	145	0	83	0	190	0
57	0	200	0	66	2	181	1
58	1	170	1	82	1	165	1
69	2	188	0	77	2	162	1
88	4	175	2	83	2	160	2
62	2	154	0	72	1	186	0
71	1	195	1	92	1	179	4
Av. 66.3	1.4	172.1	0.63	79.5	1.4	174.1	1.1
Method 3: A.A.C.C. HCl–mineral oil–gasoline							
51	2	110	0	60	1	140	2
56	1	120	1	58	1	138	1
46	6	130	0	57	2	131	4
55	1	124	3	66	1	127	1
71	2	125	2	56	2	145	2
68	5	113	1	76	6	142	0
62	2	133	2	60	1	146	0
51	3	134	1	61	2	138	1
Av. 57.5	2.8	123.6	1.3	61.8	2.0	138.4	1.4

CONCLUSIONS

(1) The pancreatin digestion (Method 1) appears to give substantially higher insect fragment recovery than either of the HCl digestion methods (Methods 2 and 3). It may be that the boiling which is necessary with HCl digestion removes some of the waxy material from insect cuticle and causes these fragments to become saturated with water and, therefore, to settle out with the plant tissue. However, the rodent hair counts were highest in Method 3 (A.A.C.C. HCl-mineral oil-gasoline). The rodent hair counts in replicate samples varied considerably, and any positive conclusion is thus inadvisable. Further comparison of the digestion methods with several types of flour seems necessary to establish positive differences.

(2) The average number of insect fragments for 16 replicates of each of the two samples when tested by two methods in which the two liter

TABLE 5.—*Comparison of filth recoveries from first and second trappings*

SAMPLE 1						SAMPLE 2					
1ST TRAP		2ND TRAP		TOTAL		1ST TRAP		2ND TRAP		TOTAL	
I.F.	R.H.	I.F.	R.H.	I.F.	R.H.	I.F.	R.H.	I.F.	R.H.	I.F.	R.H.
Method 1: F.D.A. pancreatin–gasoline–Wildman trap flask											
60	0	5	0	65	0	141	1	9	0	150	1
60	1	3	0	63	1	137	0	8	0	145	0
54	0	3	0	57	0	189	0	11	0	200	0
52	1	6	0	58	1	160	1	10	0	170	1
64	2	5	0	69	2	176	0	12	0	188	0
83	3	5	1	88	4	165	2	10	0	175	2
59	2	3	0	62	2	145	0	9	0	154	0
70	1	7	0	77	1	181	1	14	0	195	1
Av. 62.8	1.3	4.6	0.1	67.4	1.4	161.8	0.63	10.4	0	172.1	0.63
Per cent total I.F. recovered in second trap: Sample No. 1 = 6.9; Sample No. 2 = 6.0.											
Method 1: F.D.A. pancreatin–gasoline–separatory funnel											
76	2	5	0	81	2	160	0	10	0	170	0
79	0	4	0	83	0	181	0	9	0	190	0
62	2	4	0	66	2	170	1	11	0	181	1
77	1	5	0	82	1	155	1	10	0	165	1
71	2	6	0	77	0	153	1	9	0	162	1
79	2	4	0	83	2	147	2	13	0	160	2
66	1	6	0	72	1	175	0	11	0	186	0
87	0	5	1	92	1	169	4	10	0	179	4
Av. 74.6	1.3	4.9	0.1	79.5	1.4	163.8	1.1	10.4	0	174.1	1.1
Per cent total I.F. recovered in second trap: Sample No. 1 = 6.1; Sample No. 2 = 6.0.											
Method 2: F.D.A. HCl–gasoline–Wildman trap flask											
37	1	4	0	41	1	88	1	4	0	92	1
47	0	5	0	52	0	115	1	11	0	126	1
44	0	4	0	48	0	110	1	7	0	117	1
62	1	3	0	65	1	104	1	11	0	115	1
54	3	4	0	58	3	113	3	13	1	126	4
59	0	4	0	63	0	126	1	5	0	131	1
58	2	6	0	64	2	116	4	14	0	130	4
46	1	2	0	48	1	120	2	9	0	129	2
Av. 50.9	1.0	4.0	0	54.9	1.0	111.5	1.8	9.3	0.1	120.8	1.9
Per cent total I.F. recovered in second trap: Sample No. 1 = 7.3; Sample No. 2 = 7.7.											
Method 3: A.A.C.C. HCl–mineral oil–gasoline–Wildman trap flask											
50	2	1	0	51	2	106	0	4	0	110	0
53	1	3	0	56	1	118	1	2	0	120	1
46	6	0	0	46	6	127	0	3	0	130	0
52	1	3	0	55	1	120	3	4	0	124	3
69	2	2	0	71	2	122	2	3	0	125	2
66	5	2	0	68	5	111	1	2	0	113	1
60	2	2	0	62	2	130	2	3	0	133	2
49	3	2	0	52	3	131	1	3	0	134	1
Av. 55.6	2.8	1.9	0	57.6	2.8	120.6	1.3	3.0	0	123.6	1.3
Per cent total I.F. recovered in second trap: Sample No. 1 = 3.3; Sample No. 2 = 2.4.											

TABLE 5—(*continued*)

SAMPLE 1						SAMPLE 2					
1ST TRAP		2ND TRAP		TOTAL		1ST TRAP		2ND TRAP		TOTAL	
I.F.	R.H.	I.F.	R.H.	I.F.	R.H.	I.F.	R.H.	I.F.	R.H.	I.F.	R.H.
Method 3: A.A.C.C. HCl–mineral oil–gasoline–separatory funnel											
58	1	2	0	60	1	137	2	3	0	140	2
57	1	1	0	58	1	137	1	1	0	138	1
57	2	0	0	57	2	128	4	3	0	131	4
63	1	3	0	66	1	126	1	1	0	127	1
54	2	2	0	56	2	141	2	4	0	145	0
75	6	1	0	76	6	140	0	2	0	142	0
57	1	3	0	60	1	144	0	2	0	146	0
60	2	1	0	61	2	135	1	3	0	138	1
Av. 60.1	2.0	1.6	0	61.8	2.0	136.0	1.4	2.4	0	138.4	1.4

Per cent total I.F. recovered in second trap: Sample No. 1 = 2.6; Sample No. 2 = 1.7.

Wildman trap flask and one liter Squibb separatory funnel were compared is as follows: Sample 1—62.5 with the Wildman trap flask, 70.7 with the separatory funnel; Sample 2—147.9 with the Wildman trap flask, 156.3 with the separatory funnel.

(3) In Methods 1 and 2, where manual stirring is employed, an average of 6.5 per cent of the total insect fragments were recovered in the second trapping. In Method 3, where the initial mixing of extraneous particles and oil is accomplished by the convection currents due to boiling the aqueous oil mixture for ten minutes, only 2.2 per cent of the total insect fragments were recovered in the second trapping.

(4) There was little difference in the amount of plant material recovered by the different methods involved in this study. However, it was observed that the pancreatin digestion of the flour slurry had to be carefully followed as outlined in the method to insure a clean, emulsion-free separation. In a similar manner, manual stirring of the oil and digested material had to be done carefully and without undue turbulence in order to avoid formation of a heavy emulsion.

REFERENCES

(1) *Trans. Am. Assoc. Cereal Chemists,* **8,** No. 2, August, 1950.
(2) *Ibid.,* **7,** No. 2, August, 1949.
(3) CORY, R., paper presented to the Pacific Northwest Section, American Association of Cereal Chemists Convention, Seattle, Wash., June 22, 1953.
(4) *Official Methods of Analysis,* 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington, D. C., 1950.
(5) *Cereal Laboratory Methods,* 5th Ed., American Association of Cereal Chemists, St. Paul, Minn., 1947, p. 230.

SOME APPLICATIONS OF RADIOGRAPHY TO THE EXAMINATION OF FOODS AND DRUGS*

By J. FRANK NICHOLSON (Division of Microbiology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

In the past two years, both industry and regulatory agencies have used X-rays for the detection of internal insect infestation in wheat and corn.

Other dry products, corn and rice in particular, have been X-rayed for defects. The detection of cracks through the endosperm of corn is important to its economical processing into certain cereal products. In this type of milling, corn is coarsely cracked and sieved over a coarse screen. The "throughs" of this screen are sent to feed; the "overs" are used for the cereal products. If the corn has cracks through the endosperm which will produce a granulation during the milling that is finer than the coarse screen mentioned above, then the milling company may suffer a considerable loss of usable raw material. The use of X-rays to detect this checking, or cracking inside the corn, before milling begins will be of value to industry.

A similar situation exists in the processing of rice.

Because of the successful use of radiographs for the detection of internal insects and other defects in wheat and corn, X-radiation was used on other materials. First of all, it had to be determined if useful radiographs of the material can be obtained. One type of product to which X-radiation would seem applicable is spices. Several spices have been X-rayed. The interpretation of the X-ray picture in some cases may prove difficult, due to the internal structure of the seed or plant tissue involved.

Fig. 1 shows an X-ray picture of cardamon. The outer husk of most of the seeds does not show. This spice has a very complicated internal structure and, from the samples examined it seems to have sustained a large amount of damage by field insects of the sucking type.

Fig. 2 is an X-ray picture of coriander. Part of the fruits show in the radiograph as two sections. This is caused by mericarps being parallel to the X-radiation in one case and rotated horizontally in the other. It can be noted that there are also all degrees of rotation between these two extremes.

Fig. 3 is an X-ray picture of mollé. In this case the complication is due not to internal but to external depressions of the seed coat, and may also be due in part to resin pockets in the outer coating of the seed.

Fig. 4 shows allspice which gives an X-ray picture very similar to cori-

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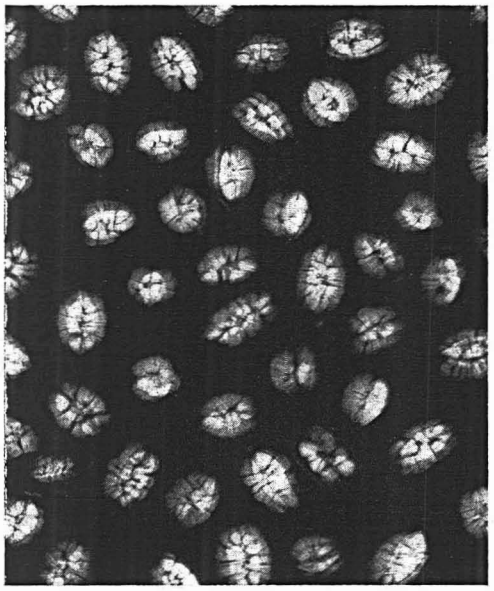

FIG. 1.—Cardamon.

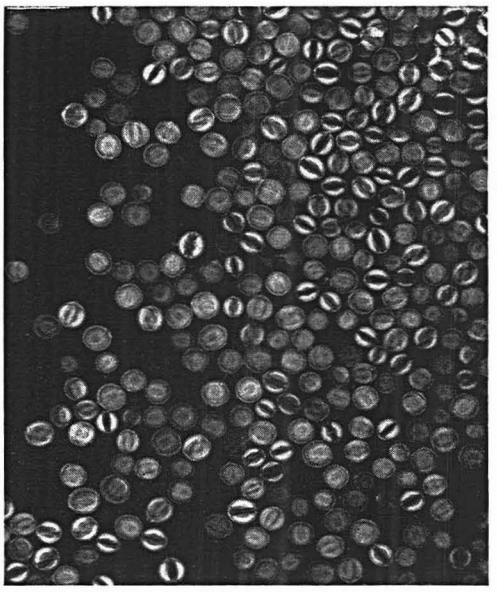

FIG. 2.—Coriander.

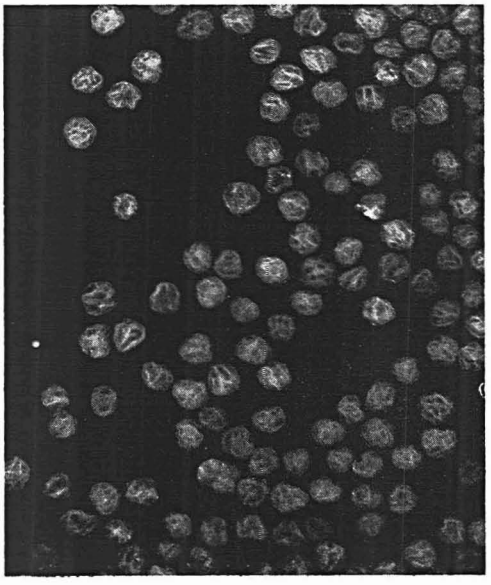

FIG. 3.—Mollé.

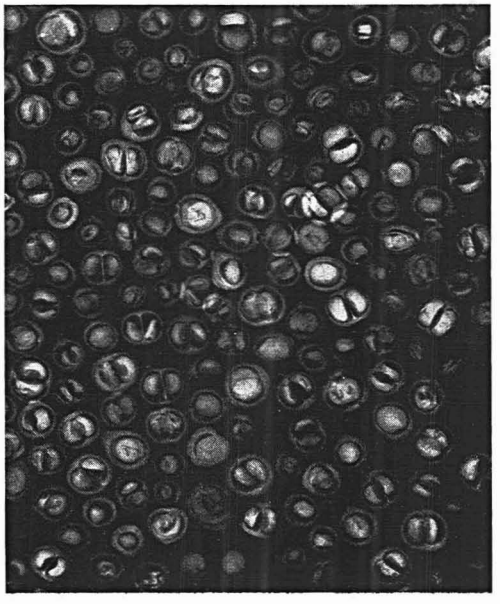

FIG. 4.—Allspice.

ander. Allspice is also a two-celled fruit and the difference in appearance in the X-ray is due to the difference in orientation of the fruit to the X-ray beam mentioned in the discussion of coriander.

Fig. 5 shows coffee containing black berries. Coffee also has a complicated internal structure. The black beans do not show sufficient contrast, compared to the normal bean, to be detected by this means. Insect infested coffee beans, however, are readily visible in the X-ray picture shown in Fig. 6.

All of these spices show complicated internal or external structural features which are hard to interpret in relation to internal insect infestation. The insect infested coffee, however, can readily be differentiated from normal coffee structure and the same may be true of some of the spices as well.

Fig. 7 shows an X-ray picture of normal black-eyed peas. There are large internal cavities in these beans which show as dark shadows near the center, introducing some complications to the interpretation of the X-ray. This complication, however, is not too serious because the gradual gradation of the internal cavities is easily differentiated from the sharp line of demarcation of the insect tunnel.

Fig. 8 shows an X-ray of rice which has some internal insect damage. Since this figure was made light in order to be suitable for projection purposes, no checking of the endosperm shows.

Fig. 9 shows extensive sucking-type insect injury to the bean. This injury took place while the bean was in a succulent condition. The insect inserted his beak into the bean and excreted a proteolytic enzyme, then withdrew the proteinaceous material. This gives rise to such an extensive depression in the bean that it somewhat resembles internal feeding insect damage as shown in Fig. 10.

Fig. 10 demonstrates various degrees of internal insect damage. The injury here varies from exit holes to small pockets due to early insect growth. It can be noted in this figure that tunnels in the center of the bean are often less dark than those closer to the side. It can also be noted that tunnels smaller in diameter in a smaller bean show darker than a larger tunnel in a larger bean. This gave rise to the idea that since this is a subtractive process, there must be a definite relationship between the thickness of the bean and the size of the insect excavation in order to register on the film. Thus, to register on the film with equal intensity, the cavity for the center of the bean must be larger than at the margin.

To test this theory and also to get an idea of the approximate size of the smallest hole which would register, a series of drill holes was made in corn. In Fig. 11 are shown the holes which become progressively larger from left to right. There is little difference in intensity from side to side of the corn and even the smallest hole used, which was 0.040 of an inch, registers well.

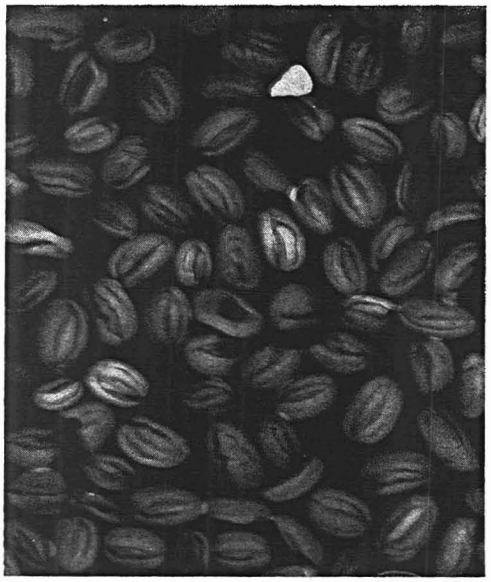

FIG. 5.—Coffee with black berries.

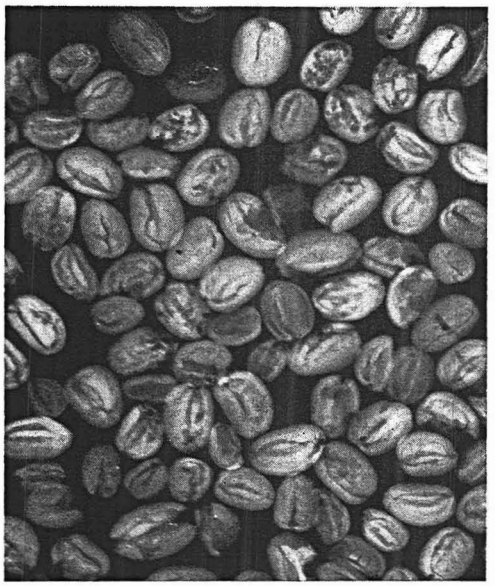

FIG. 6.—Coffee showing insect infestation.

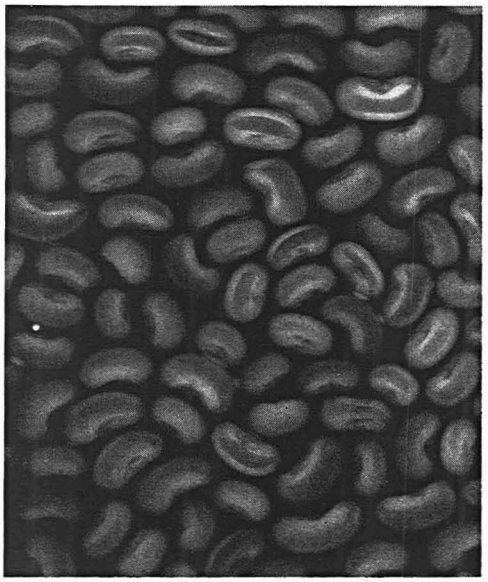

FIG. 7.—Black-eyed peas.

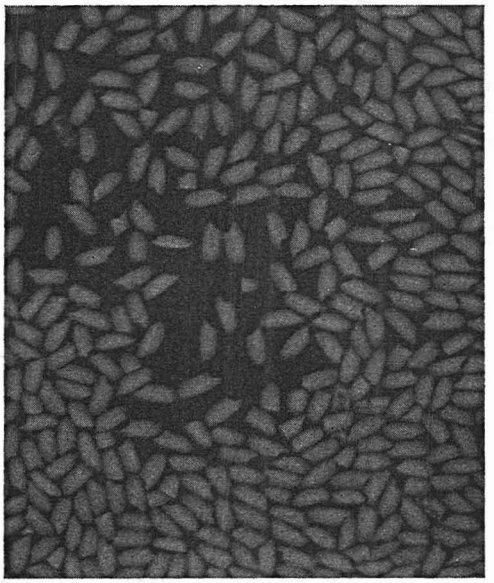

FIG. 8.—Rice.

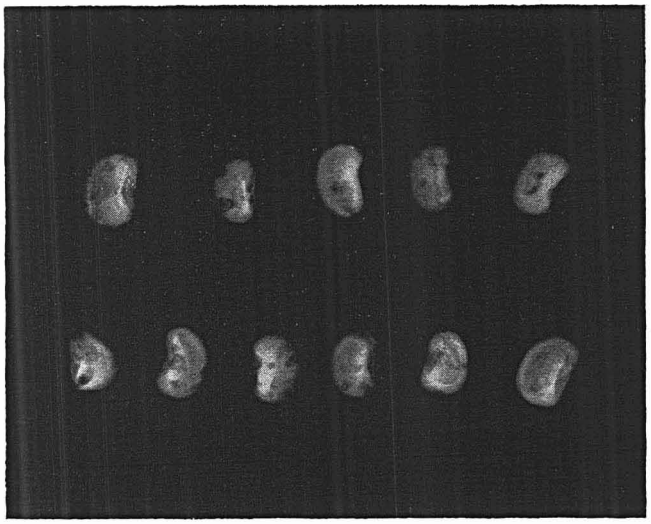

FIG. 9.—Insect-damaged beans.

Fig. 12 shows some black-eyed peas drilled with this same set of drills. It can be noticed here that some of the drill holes fail to show in the center of the bean. In this case the size of the beans has more influence on whether or not the holes show than does the size of the hole. Beginning with the two beans turned horizontally in the second row, the holes are drilled parallel to the X-ray beam. It is interesting to note that a very

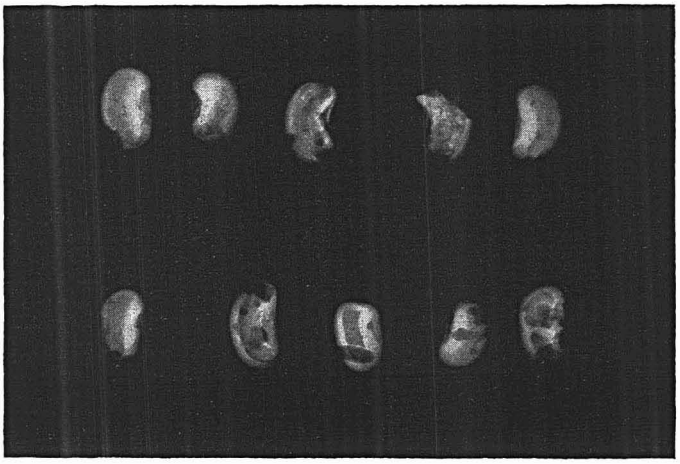

FIG. 10.—Internal insect damage.

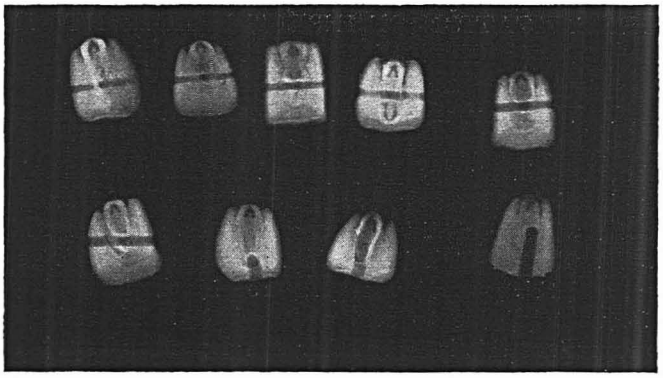

FIG. 11.—Corn, showing drill-holes.

slight deviation from the parallel makes the holes appear oval, and slightly farther over, makes the hole show as a line.

The figures give some idea of the complicating features of some of the seeds and other plant structures. The preparation of insect infested material and the interpretation of the radiograph is slow and will require considerable study. However, the possibilities of X-ray methods are numerous and should be explored as soon as possible.

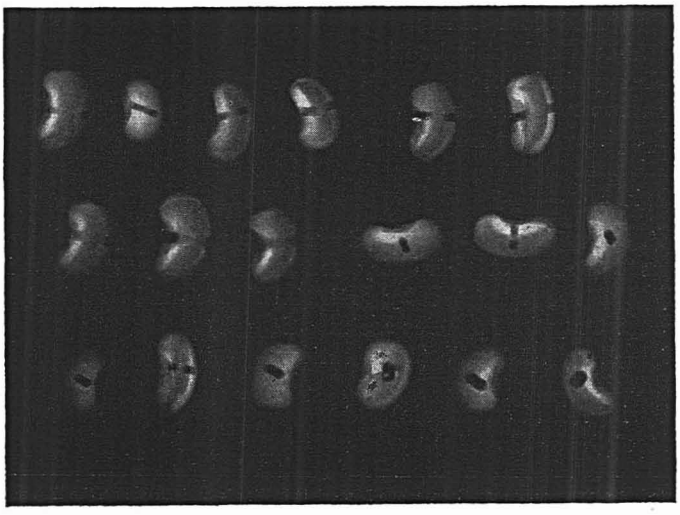

FIG. 12.—Black-eye peas showing drill holes.

IDENTIFICATION OF INSECT FRAGMENTS IN CEREAL PRODUCTS*

By W. H. SCHOENHERR (Lauhoff Grain Co., Danville, Ill.)

(Mr. Schoenherr illustrated his paper with a series of 92 color projections, and pointed out that any discussion of insects and insect fragments is best handled with visual aids. Forty-one of these slides were furnished by the Food and Drug Administration, fourteen by the Kroger Food Foundation, and thirty-seven were prepared by the author's own company. Unfortunately, due to cost and technical difficulties, it is not feasible to reproduce this large number of color slides here.† A synopsis of this paper is presented.—Editor's Note.)

The insects which cause damage to grain and cereal products are grouped as follows:

Group 1. Insects that feed primarily within grain:
- (*a*) Granary weevil
- (*b*) Rice weevil
- (*c*) Angoumois grain moth

Group 2. Insects that feed in both grain and cereal products:
- (*a*) Cadelle
- (*b*) Lesser grain borer

Group 3. Insects that feed primarily on cereal products.
- (*a*) Confused flour beetle
- (*b*) Saw-toothed grain beetle
- (*c*) Flat grain beetle
- (*d*) Indian meal moth
- (*e*) Mediterranean flour moth
- (*f*) Rice moth
- (*g*) Mites

Group 4. Insects that "stray" into milling machinery or into cereal products but are not ordinarily considered mill pests. (All insects that fly and are attracted by artificial lights could be included in this group. Only four species are mentioned.)
- (*a*) Black carpet beetle (Dermestid)
- (*b*) Silverfish
- (*c*) Springtail (Collembola)
- (*d*) Book louse (Psocid)

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* Presented at a symposium on Extraneous Materials in Foods and Drugs at the Annual Meeting of the Association of Official Agricultural Chemists, Oct. 13, 1953, at Washington, D. C.

† Copy of the original text may be obtained from the author. Arrangements may also be made for loan of the slides.

The Group 1 insects cause most damage. The slides depicted some insects of all groups, usually in various stages of their metamorphosis, with examples of the damage they cause.

A second aspect is the type of fragments caused by these insects. These fragments may be from adult insects, larvae, pupae, head capsules, pupa cases, larva heads, mandibles, antennae, elytra, legs, etc.

The ability to distinguish between foreign plant material (hairs, seed coats, etc.) and insect fragments is important. The use of good visual aids is very helpful. Slides were shown which depicted various characteristic landmarks of insect fragments, such as mandible teeth, segmentation, pattern caused by elevations and depressions on the surface, setae, seta pits, over-all pitted appearance, and presence of other characteristic structures. A knowledge of such characteristics permits identification of the fragments as such and also of the specific insect type which contributes the fragment.

REFERENCES

(1) *Microanalysis of Food and Drug Products*, Food and Drug Circular #1, U. S. Government Printing Office, Washington, D. C.
(2) HARRIS, K. L., *This Journal*, **35**, 898 (1950).
(3) KURTZ, O. L., CARSON, N. A., and VAN DAME, H. C., *ibid.*, **38**, 817 (1952).
(4) *Stored Grain Pests*, U. S. Department of Agriculture, Farmer's Bulletin 1260 (Revised, 1953).

INSECT CONTAMINATION IN SUCCULENT VEGETABLES*

By T. H. McCORMACK (Kroger Food Foundation, Cincinnati, Ohio)

Because of their nature, succulent vegetables, particularly the leafy varieties which contain folds and crevices, are difficult to clean. Most consumers can tolerate a little sand with their spinach, since they realize what a laborious task it is to completely separate the edible from the inedible. Sand is not especially repulsive to the palate, and we know that that it is practically impossible to eat foods completely free from this material. Other extraneous materials, more distasteful than sand, may be present in these folds and crevices, however.

Canned greens are sometimes contaminated with plant lice or aphids, ladybugs, stink bugs, cucumber beetles, flies, spiders, cutworms, cabbage web worms, and ants. Naturally, every effort should be made to exclude insects from food products, but in spite of this, it is often possible to recover one or more insect types from canned greens.

* Presented at the Sixty-seventh Annual Meeting of the Association of Official Agricultural Chemists Oct. 12, 13, and 14, 1953, at Washington, D. C.

A modified flotation method is used to extract the light filth from canned greens. It consists of boiling the sample, which has been previously chopped into small pieces, in a solution containing lead acetate, acetic acid, and water. After the sample is cooled, flotation is performed in a Wildman trap flask; gasoline is used to attract the extraneous material. The liquid from the can is also examined by means of flotation.

Space does not permit a discussion of each type of insect mentioned in the list above, but it is possible to consider three of the more common ones, namely, the aphid, the ladybird or lady beetle, and the fly.

APHIDS

Aphids are soft-bodied "plant lice" as large, on the average, as pinheads. They are usually green in color although some are brown, yellowish, pinkish, or black. They feed by thrusting sharp hollow stylets from their beaks in among the cells of the plant and sucking out the sap. This causes blighting of buds, dimpling of fruits, curling of leaves, or the appearance of discolored spots on the foliage. As the aphids become more abundant, the plants generally wilt from loss of sap and possibly from being poisoned by the saliva of the aphid.

Aphids winter as fertilized eggs on some perennial plant or dead remnant of annual vegetables. When the weather becomes warm enough, small nymphs hatch from the eggs and grow to full size, but never get wings. Each of these aphids is the start of a colony of aphids and is called a *stem-mother*. All are female and reproduce without mating. The young are born ovoviviparously, or already hatched from the egg. Toward the end of the season, winged males as well as winged females are produced. These females cannot reproduce unless they mate. The female produces one or more large eggs and the stem-mothers hatch from these eggs in the spring.

The aphid is identified by its soft oval-shaped body, long legs and antennae, and two short projections on the posterior. Cast skins, which are the shells discarded by nymphs passing from one stage to another, can also be found in canned greens. These are transparent, hollow, and ghost-like in appearance.

THE LADYBIRD

The ladybird, or lady beetle, helps to control the aphids. In the larva stage, this beetle may destroy aphids at the rate of one a minute over a considerable period of time. The adult ladybird is yellow or orange in color with a number of black spots on the elytra.

THE HOUSE FLY

The house fly is a very common insect and is found throughout the world. Since the adult is practically omnivorous, the insect or parts of it may be recovered in a wide variety of food products. The adult has a keen sense of smell and can quickly locate food in and around food processing plants and canneries. The female frequently deposits eggs in decayed fruits and vegetables. An almost ideal breeding place is a plant or cannery where refuse is allowed to accumulate. If the fly's entrance into the plant is not prevented, the female may deposit eggs in the material to be canned or processed. The eggs develop into larvae in eight to thirty hours; therefore, if there is sufficient delay before foods are packed, larvae will develop. These eggs or larvae (maggots) can be recovered from canned or bottled foods.

The eggs of the fly are about $\frac{1}{16}$ inch in length; 100 to 150 of them are laid at one time, and the average production per fly is about 500. These eggs hatch out into small larvae in eight to thirty hours. These maggots are white with one pointed and one blunt end. The pointed end contains the mouth hooks which are black and readily visible through the semi-transparent skin. After a period of 5 to 14 days, during which the larva reaches a length of $\frac{1}{3}$ to $\frac{1}{2}$ inch, the puparium is formed. The puparium is brown and oval-shaped; in this puparium the adult develops in three to ten days. After about $2\frac{1}{4}$ to 23 days, the female may lay eggs and begin another cycle.

REFERENCES

(1) METCALF, C. L, and FLINT, W. P., *Destructive and Useful Insects*, McGraw-Hill Book Co., Inc., New York, 1939.
(2) *Insects*, The Yearbook of Agriculture, U. S. Department of Agriculture, Washington, D. C., 1952.
(3) *Filth in Canned Greens (Revised)*, M 13K, National Canners Association, Washington, D. C., September, 1945.

EXTRANEOUS MATERIALS IN SPICES*

By T. H. McCORMACK (Kroger Food Foundation, Cincinnati, Ohio)

Spices have been used since ancient times, and sanitation in the countries producing them has improved little since then. The bulk of the spices used in this country are imported from the Orient from such places as Sumatra, New Guinea, Indo-China, and others. Because primitive

* Presented at the Sixty-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 12, 13, and 14, 1953, at Washington, D. C.

ideas of cleanliness sometimes prevail, it is not surprising that spices coming from these countries may contain excessive extraneous material.

Dealers in this country usually expend great effort to remove filth from spices, and are quite successful in most cases. There are some spices, however, that resist all cleaning methods; an outstanding example is celery seed. It has been difficult to obtain satisfactory lots for the past two or three years because of the high concentration of heavy filth in both French and Indian spices.

Spices are processed for microscopic examination of light filth according to types. The light filth is recovered from all spices by means of flotation in the Wildman trap flask, but spices of high fat content are first defatted in a fat extractor. All are boiled for a period of time in a dilute hydrochloric acid solution and cooled before flotation, but in the case of some herbs, lead acetate and acetic acid are added before boiling.

Sedimentation is the method used to extract heavy filth such as sand and dirt particles, excreta fragments, etc. A mixture of carbon tetrachloride and chloroform is used. It is of such density that it will permit the heavy filth to fall to the bottom of a conical beaker, but will cause the spices to float.

Many of the insects that infest spices and herbs are the same as those that attack stored grain and other dry food products. Four of those frequently encountered are the cigarette beetle, the confused flour beetle (and rust-red flour beetle), the sawtoothed grain beetle, and the Indian meal moth. A brief description accompanying the illustrations might be helpful in the identification of these insects.

The *cigarette beetle* (Fig. 1) can be found in such materials as black and red pepper and condimental seeds. This beetle is white as a pupa, yellowish-white with a light brown head as the larva, and light brown in color in the adult stage. The adult has a hump-backed appearance and is covered with fine hairs or setae. These beetles pupate in silken cocoons.

It is interesting to note that these insects are very hardy and can subsist for long periods under adverse conditions. A number of the insects developed from eggs on some red peppers or *chilies* kept in a small sealed display jar. After three years there are still live insects in the jar. In spite of the fact that the peppers were dried and consequently contained little moisture, and that no additional oxygen entered the jar, the life cycle was repeated many times during the period, a fact verified by the layer of dead adults and the frass and excreta in the lower portion of the jar.

The *confused flour beetle* (Fig. 2) may feed on red pepper, ginger, and other spices. The adults are very active, red-brown in color, and are known among millers as "bran bugs." The pupae are white; the larvae are brownish-white worms and are longer than the other two stages, which is the case with most beetles.

The *sawtoothed grain beetle* (Fig. 3) has been found in dehydrated onion

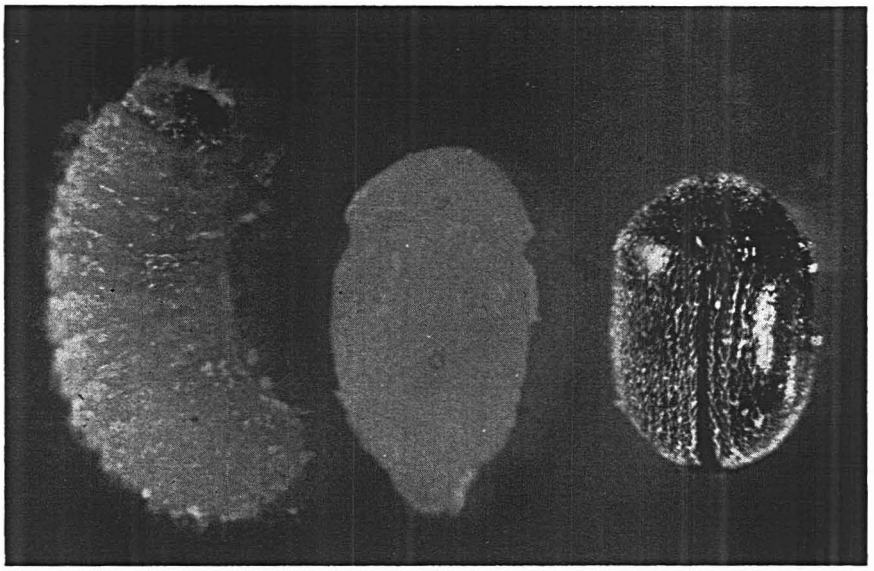

FIG. 1.—Cigarette beetle: larva, pupa, and adult.

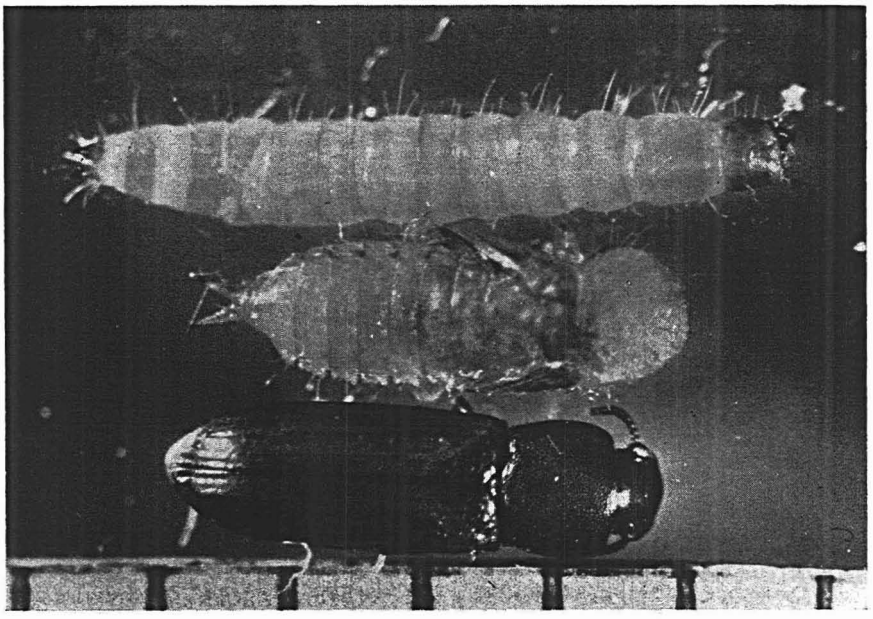

FIG. 2.—Confused flour beetle: larva, pupa, adult (with metric rule).

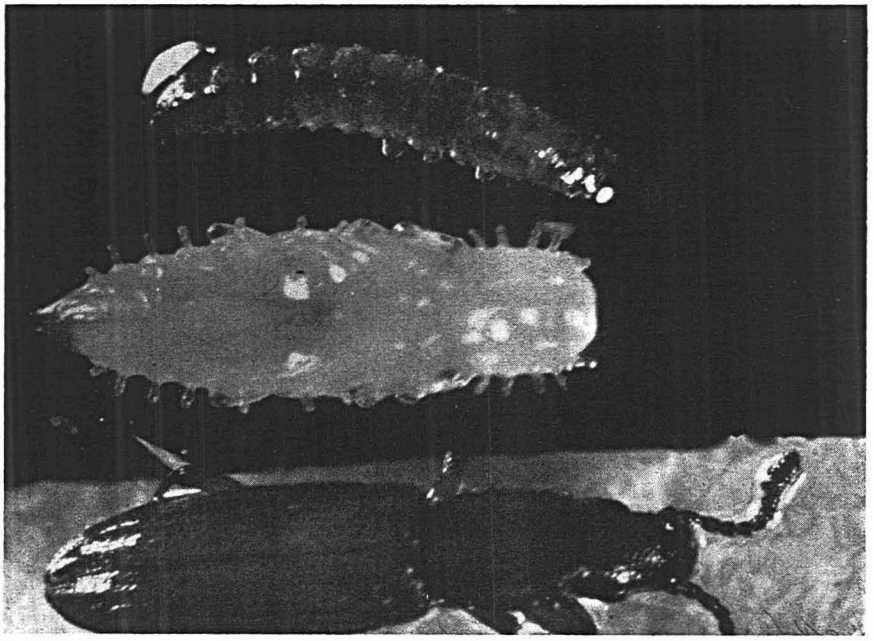

FIG. 3.—Sawtooth grain beetle: larva, pupa, adult.

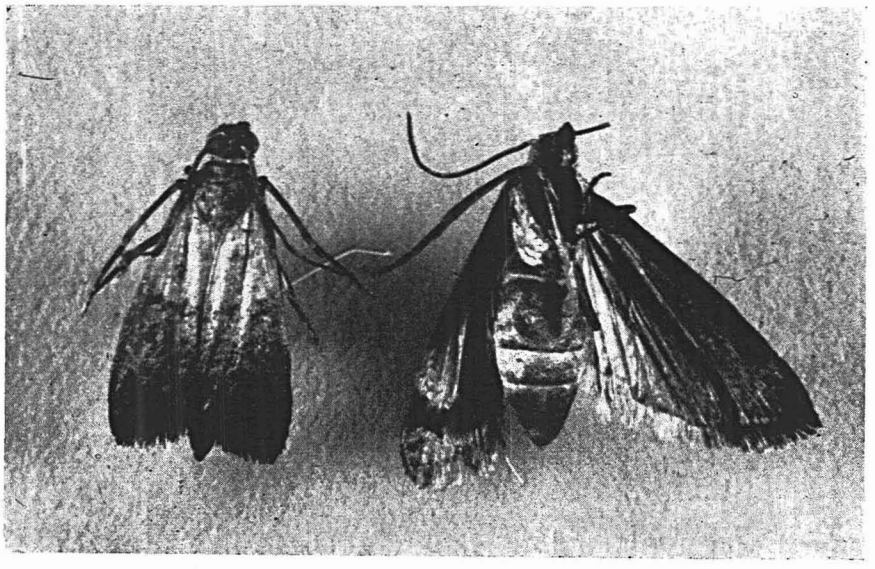

FIG. 4.—Indian meal moth, adult (top and bottom views).

flakes and will subsist on many other dry spices. The adult and pupa are distinguished by the presence of tooth-like projections along both edges of the prothorax; however, the adult is dark brown while the pupa is white. The larvae are white with brown heads.

The *Indian meal moth* prefers herbs, and the adult can frequently be seen fluttering around where these products are stored. The adult (Fig. 4) has a grayish-white stripe across its body, separating two stripes of reddish brown. The larvae are usually white in color but often have a distinct greenish or pinkish tinge. They pupate within a thin silken cocoon.

Insects fragments and excreta pellets from these insects, as well as other contaminants, are frequently found in spices. The following are some examples of extraneous materials recovered by flotation, sedimentation, and macroscopic examination:[1]

(1) Insect fragments from stick cinnamon.
(2) Silken cocoon in stick cinnamon.
(3) Rodent and insect excreta, sand, and dirt particles from celery seed.
(4) Sand, dirt particles in stick cinnamon.
(5) Mite.
(6) Scales from Indian meal moth.
(7) Larva from celery seed.
(8) Wood fragments from cream of tartar.
(9) Insect excreta pellets from dehydrated onion flakes, sawtoothed grain beetle.
(10) Fossilized seashell fragments from French celery seed.
(11) Insect excreta pellets from leaf marjoram.
(12) Insect fragments in leaf marjoram.
(13) Elytrum in leaf marjoram.
(14) Insect fragment from parsley flakes.
(15) Rodent guard hair in parsley flakes.
(16) Larva from marjoram, black carpet beetle.
(17) Larva fragments from marjoram, black carpet beetle.
(18) Moldy whole black pepper.
(19) Moldy whole black pepper, germinated.
(20) Same as No. 19 at higher magnification.
(21) Mossy growth on Saigon stick cinnamon.
(22) Indian meal moth puparium and webbing.

REFERENCES

(1) GIBBS, W. M., *Spices and How to Know Them*, The Matthews-Northrup Works, Buffalo, New York, 1909.
(2) METCALF, C. L., and FLINT, W. P., *Destructive and Useful Insects*, McGraw-Hill Book Co., Inc., New York, 1939.
(3) *Insects*, The Yearbook of Agriculture, U. S. Department of Agriculture, Washington, D. C , 1952.

[1] Because of expense and limitations of space, subsequent illustrations have been omitted.—Ed.

MOLD AND ROT IN TOMATO CATSUP*

By T. H. McCormack (Kroger Food Foundation, Cincinnati, Ohio)

TOMATO HISTOLOGY

Mold counting, by means of the Howard mold counting method, is the test most widely employed to determine both the condition of the raw materials used in tomato products and the cleanliness of the methods used by the packer. Depending on the type of mold found, a high count

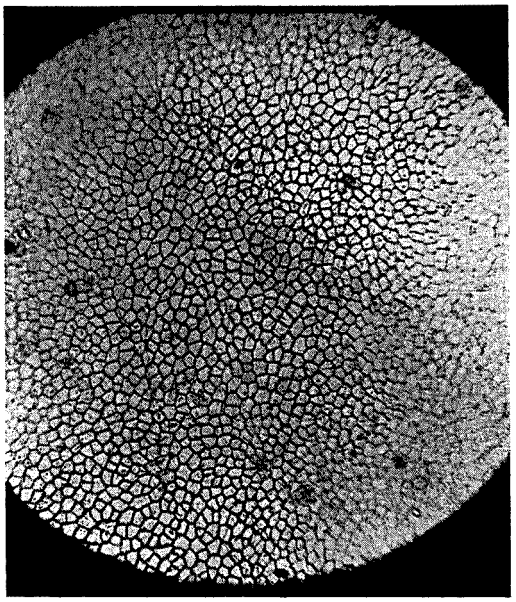

FIG. 1.—Skin cells of the tomato.

will indicate that rotten tomatoes were used or that the product was packed in an unsanitary plant.

In order to obtain a reliable count, the technician must have some knowledge of mold, and there are a number of properties possessed by mold filaments which aid the technician in its identification. However, it is noted that mold is not the only object observed on the Howard cell and some of the objects encountered bear a considerable resemblance to mold. Thus it might be well to examine the microscopic field to see what else might be present. In catsup, the only insoluble materials other than mold are parts of the tomato (and spices); therefore it is appropriate to examine the histology of the tomato first.

* Presented at the Sixty-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 12, 13, and 14, 1953, at Washington, D. C.

The tomato[1] is made up of stem cells, flesh cells, fibrovascular bundles, seed covering cells, skin cells, seed cavity lining cells, seed hairs, seed cells, core cells, and the jelly-like mass surrounding seeds.

Skin Cells (Fig. 1), are greenish-yellow in color and polygonal in shape. The cell walls are rather thick, and amber in color. Under 100 × magnification, a piece of tomato skin resembles hammered metal.

FIG. 2.—Flesh cells of the tomato.

Flesh Cells (Fig. 2), are larger than the skin cells and tend to be more oval-shaped. They are thin-walled, and those just beneath the skin and next to the seed cavity are smaller and narrower. Although they are transparent, bits of cellular material can be seen. Care should be employed not to confuse the walls of ruptured cells with mold hyphae, since the edge of a cell appears as a line under the microscope.

Seed Cavity Cells (Fig. 3), form the lining of the hollow enclosing the seed. They are smaller than flesh cells and irregular in shape, and might be compared to the pieces of a jig-saw puzzle with interlocking edges. They are almost completely transparent with some flecks of cellular material.

[1] (Because of expense and limitations of space, a number of illustrations have been omitted.—Ed.)

Fibrovascular Bundles have the appearance of coiled springs with white thread-like veins. In catsup or a similar product, the bundles are frequently broken; the coils are released in larger spirals and in some smaller coiled fragments. These should not be confused with mold filaments since the coiled effect and roughly broken ends do not occur in mold.

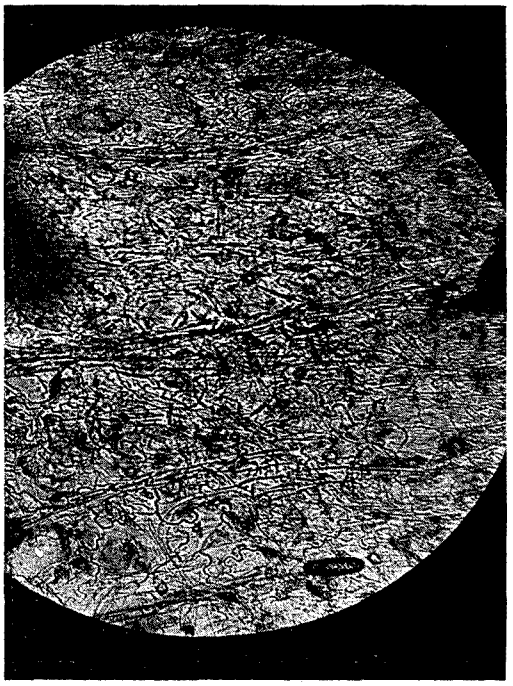

FIG. 3.—Seed cavity cells of the tomato.

Seed Covering Cells have indefinite outlines and are arranged in various patterns of which the "starlike" pattern is the most common. These cells compose the skin to which seed hairs are attached.

Seed Hairs ordinarily are found attached to the seed or part of a seed, but occasionally a free one will be seen. The free ones should not be mistaken for mold filaments, since they are tapering and thin-walled, and the broken ends are rough or jagged. In addition, hairs usually have a bluish tinge.

Core Cells have a rectangular shape and occur in parallel rows at the center of the tomato, especially near the stem and blossom ends.

Jelly-Like Mass Surrounding Seeds. The cells are globular to spherical with granular contents. The nuclei are very conspicuous.

MOLD

Mold hyphae generally occur in comminuted food products without fruit bodies, and in most cases this renders impossible the identification of the specific kind of mold. This is unimportant, however, since the technician is only concerned with whether a filament is mold or some histological tomato fragment.

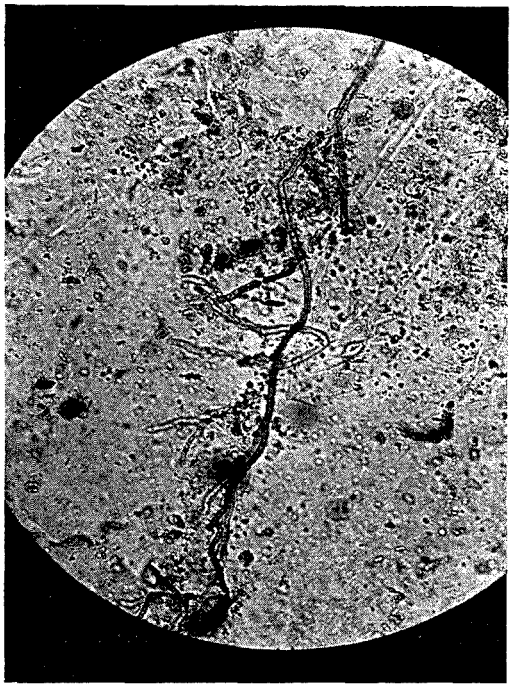

FIG. 4.—Mold filament with granulation.

The Howard mold count method employs a Howard cell which is designed to hold a minute quantity of the material to be examined. In the case of tomato catsup, the sample is thoroughly mixed and a quantity sufficient to cover the central disc is placed on the cell with the aid of a scalpel and dissecting needle. By means of a microscope supplying a magnification of about 100 × and a field of specific size, twenty-five fields are examined for mold filaments and the number of positive fields (those containing mold hyphae of adequate length) are totaled. Two or more slides should be counted until reasonable agreement is attained. The result is calculated as per cent positive fields by using an appropriate formula.

To identify mold, the following properties should be considered:

Parallel Walls.—Mold hyphae are tubular but usually appear flat under the microscope, and since the tubes are uniform, the walls will seem parallel. Two exceptions are *Mucor* and *Oospera*, where tapering occurs.

Granulation.—Living protoplasm within the mold hyphae gives a granular appearance to it. This property may still exist even after the mold is killed by processing, but in some cases this granular material coagulates into a transparent material. *Mucor* and *Rhizopus* have prominent granulation (Fig. 4).

Septation.—Most mold filaments contain cross walls which separate the tubes into section. This serves as a means of identification. The cross walls are not present in *Mucor*.

Branching.—If the filaments are not too short, the presence of branching can be used as an aid in the positive identification of hyphae.

A filament may be considered as mold if it possesses one of the following:

(*a*) Parallel walls of even intensity with both ends blunt.
(*b*) Parallel walls of even intensity with branching.
(*c*) Parallel walls of even intensity with granulation.
(*d*) Parallel walls of even intensity with septation.
(*e*) Parallel walls of even intensity with one end blunt and one rounded (occasionally).
(*f*) Gradually tapering walls of even intensity; characteristic granulation or septation.

ROT FRAGMENTS

A high mold count is evidence of decomposition, but a low mold count does not necessarily prove that the product was made from wholesome tomatoes. Frequently tomatoes contain rot but few mold filaments are found. In these cases fragments of rotten tomato can be found distributed throughout the product. The following method is used to determine the number of rot fragments per gram:

A small sample of catsup is stained with saturated gentian violet solution and measured quantities of this are put on two specially designed counting plates. Each plate is examined under a wide-field microscope; relatively low magnifying power and transmitted light are used. A rot fragment is identified as a clump of opaque or semi-opaque material (rot) with a periphery of mold filaments. By totaling the number on both plates and multiplying this sum by a factor, the number of rot fragments per gram of original sample are calculated. An excessive number indicates that rotten tomatoes were used to make the product.

REFERENCES

(1) TROY, U. S., *Mold Counting of Tomato Products*, Research Division, Continental Can Co., Chicago, Illinois, 1952.
(2) BURNS, E. E., and DESROSIER, N. W., *The Canner*, July 28, 1953.
(3) *Rot Fragments in Tomato Products*, M 13D (Revision), National Canners Association, Research Laboratories, September, 1943.

AN APPROACH TO INSECT LARVAL IDENTIFICATION BY STRUCTURES ISOLATED AS FRAGMENTS FROM CEREAL PRODUCTS*

By O. L. KURTZ (Division of Microbiology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

The type of insect fragments which contaminate cereal products depends on the insect types infesting the raw product and on the insects which may infest the mills and bakeries in which the products are produced. Once the flour, baked products, or alimentary pastes have been manufactured, any comminuted contamination present cannot be removed. Thus, the extent of contamination is determined by counting the recovered insect fragments; the source of contamination can be determined by specifically identifying many of these same fragments. Specific identification of some recovered fragments is essential to determine whether the product was contaminated as such, or whether the contamination stems from an infestation at an earlier stage.

In brief, insect infestation in cereal products leaves sufficient evidence to serve as indices of previous field, storage, mill, or bakery conditions. The determination and identification of insect materials discussed by Harris (1) and by Kurtz, Carson, and Van Dame (2) will illustrate the basic problems of insect fragment identification and the use of the morphology of a body structure (mandible) to differentiate some common storage insects. Much of the basic insect morphology used in insect fragment identification is discussed by Harris (1) and is covered more completely by Snodgrass (3).

The insect body is reinforced by an outer covering, the cuticula, which becomes sclerotized or hardened in certain areas to form body wall plates or sclerites. These characteristically-shaped sclerites constitute the insect exoskeleton and are the basis for fragment identification. The various types of sutures that delimit the boundaries of the body sclerites, the pits and ridges which serve as points of attachment for muscles and points of articulation for appendages, the varied surface patterns, the setal

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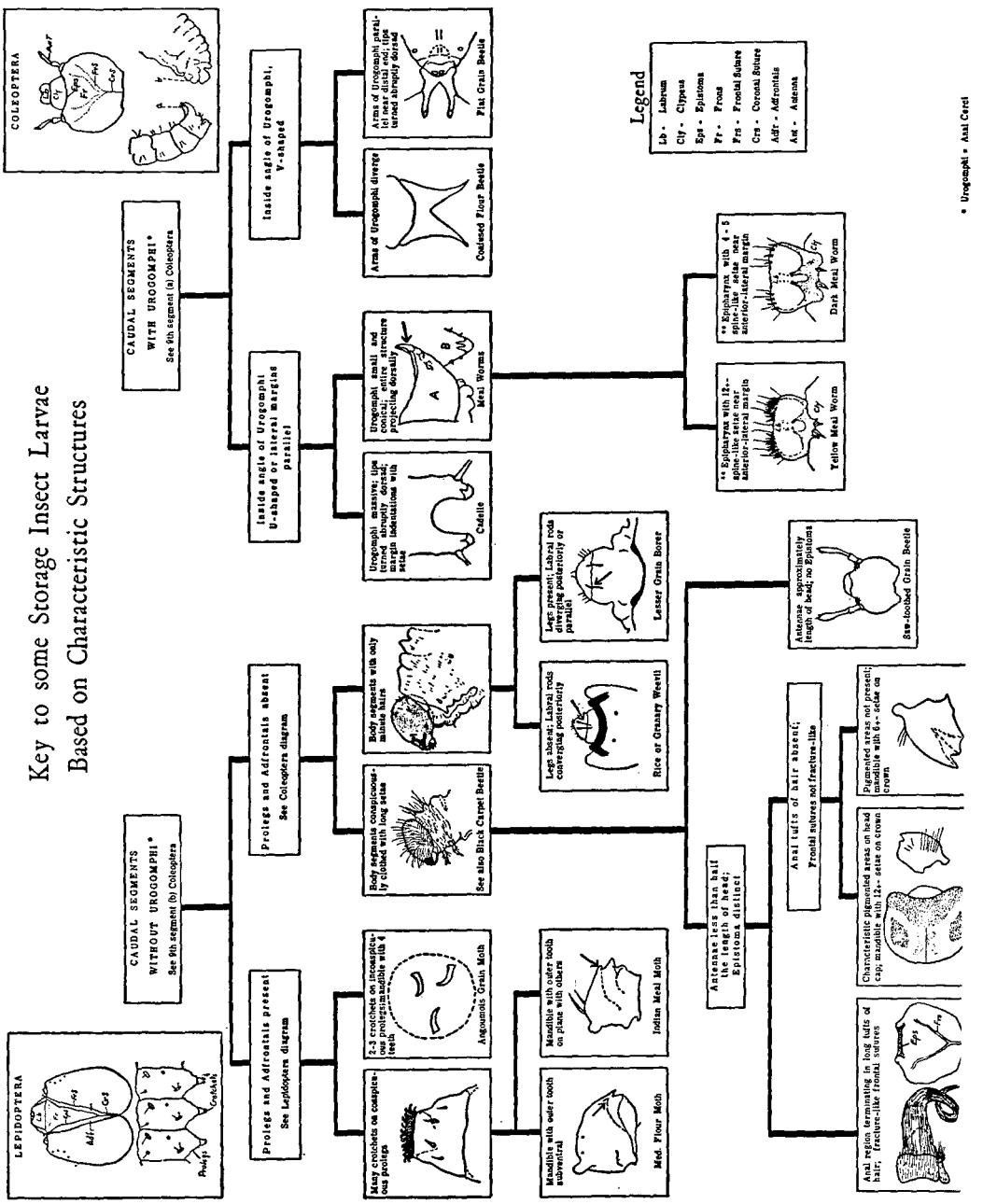
FIGURE 1

Key to some Storage Insect Larvae
Based on Characteristic Structures



types and arrangements, and the pigmentation all produce a variable assortment of morphological characteristics. These, by their own complexities, serve to identify and differentiate insects by means of their recovered fragments.

Unlike the adult exoskeleton, the larval covering is usually soft and flexible, and the sutures fusing the head sclerites and other body segments are comparatively weak. When larval forms are subjected to a grinding process, the exoskeleton does not shatter into nondescript pieces but tends to flatten and fracture along definite sutural lines. Thus, many discrete structures and sclerites retain their characteristic forms and diagnostic features. For example, certain sclerites of the head (frons, geni), certain mouthparts (labrum, mandibles), and certain abdominal appendages (anal cerci, pseudopods) usually maintain all or a substantial part of their characteristic form. The characteristic shape, pigmentation, surface patterns, setal types and arrangements, and suture orientation of these same structures serve to identify and differentiate the insect or insects in question.

This paper deals only with the immature (larval) forms and illustrates certain generalized characteristics of two insect orders which are responsible for the bulk of cereal contamination (Fig. 1). Using larval forms, the key (Fig. 1) deals with certain generalized structures characteristic of these orders and with certain selected structures specific for individual species, discrete portions of which break from the larval exoskeleton and may be isolated as fragments from cereal products.

The key is especially designed for use in routine analysis and makes use of certain outstanding characteristics. The illustrations cover most of the characteristics used in the word-description couplets. The key does not refer to all species which may be encountered, but does consider those that most commonly infest cereal products.

Other differentiating features which could be used have not been discussed.

REFERENCES

(1) HARRIS, K. L., *This Journal*, **33**, 898 (1950).
(2) KURTZ, O. L., CARSON, N. A., and VAN DAME, H. C., *This Journal*, **35**, 817 (1952).
(3) SNODGRASS, R. E., *Principles of Insect Morphology*, McGraw-Hill Book Co., New York, 1935.

INSTRUCTION IN MICROANALYTICAL METHODS*

By HOWARD R. SMITH (National Canners Association, Washington, D. C.)

Methods used in the examination of foods are developed through investigation of the various avenues of approach which show promise of contributing to a solution of the particular problem. Complex research tools may be utilized. The collaborative studies which are so essential to the development of a satisfactory method nearly always reveal needed changes if the method is to be carried out in the same manner and with the same degree of satisfaction by all properly trained analysts who may have occasion to use it. Research and trial are necessary steps in the development of a method before it can be designated an official method to be used in testing foods for compliance with the laws.

When a method is established as official, the situation changes. The method must thereafter be applied exactly as written. Even a slight change cannot be made except through the formal procedures for modification. This discussion has to do only with instruction in carrying out the official procedures.

Experience has established that some of the official microanalytical procedures cannot be thoroughly mastered without personal instruction in their details. An example of this is the Howard mold count (1) as applied to tomato products. Certain details of technic in carrying out this official procedure have been generally accepted and have been written down for the guidance of analysts (2, 3). But no matter how much training and experience an analyst may have had in other microanalytical technics, he needs personal instruction in the details of the official Howard mold count method before he is able to obtain results that can be interpreted on the basis of the information obtained over the past years (4).

With proper instruction it is possible for analysts who do not have a broad scientific training to learn the details of procedure and to carry out the method in a satisfactory manner. The Howard mold count method was first presented to industry in 1916 by Dr. W. D. Bigelow, who acted as Associate Referee on Vegetables (4). Since then many hundreds of analysts have learned and applied the method. Manufacturers of tomato products employ analysts during the canning season to make mold counts of batches during manufacture. Since this is seasonal work, many new analysts must be employed each year. The instruction of these analysts in the details of the method is a regular part of the preparation for the canning season.

Where the entire time of the instructor can be devoted to one or two

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students it is not necessary to have special technics for the instruction. However, some of us have been called on each year to give instruction to large groups of students, many of whom have not had previous training with the microscope. A series of lectures, demonstrations, and special studies, covering a period of eight days, has been developed for the training of such analysts. The studies include the following points:

(1) The construction, care, and use of the compound microscope.
(2) The histology of the tomato fruit.
(3) The characteristics of the mold filaments.
(4) The construction and use of the special Howard mold count cell.
(5) The details of technic of the official Howard mold count method.
(6) Special studies on the identification of mold filaments.
(7) Special difficulties inherent in the nature of the product to be examined.
(8) Interpretation of the results.

PERMANENT MOLD COUNT SLIDES

The most essential and difficult part of the method is the discovery and identification of mold filaments. In order to study this feature most intensively, special permanent slides have been developed (5, 6). A sample of tomato product is thickened so that the filaments will not change position on the slide, and particular fields to be examined are designated with exactitude.

A pattern for indicating 25 fields to be examined is made by punching holes in thin, colored, transparent cellophane. Each hole is, as nearly as possible, 1.38 millimeters in diameter. This pattern is permanently cemented to a microscope slide under a regular microscope cover slip with Canada balsam, and after thorough drying and cleaning, becomes a "pattern slide." A sample of tomato product is carefully mixed with an equal volume of warmed U.S.P. glycerine jelly in such a manner that air bubbles are avoided. While this mixture is still warm, a drop of it is

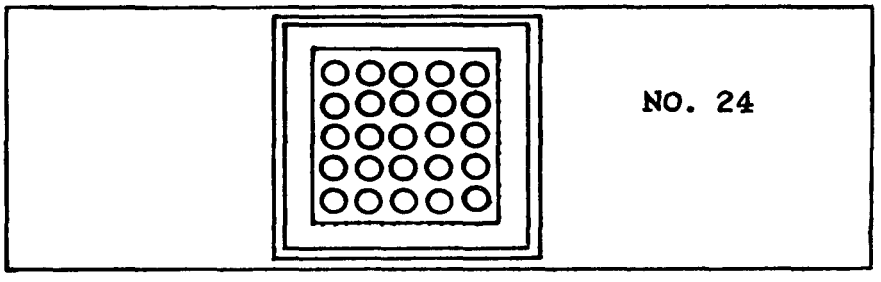

FIG. 1.—Permanent mold count slide.

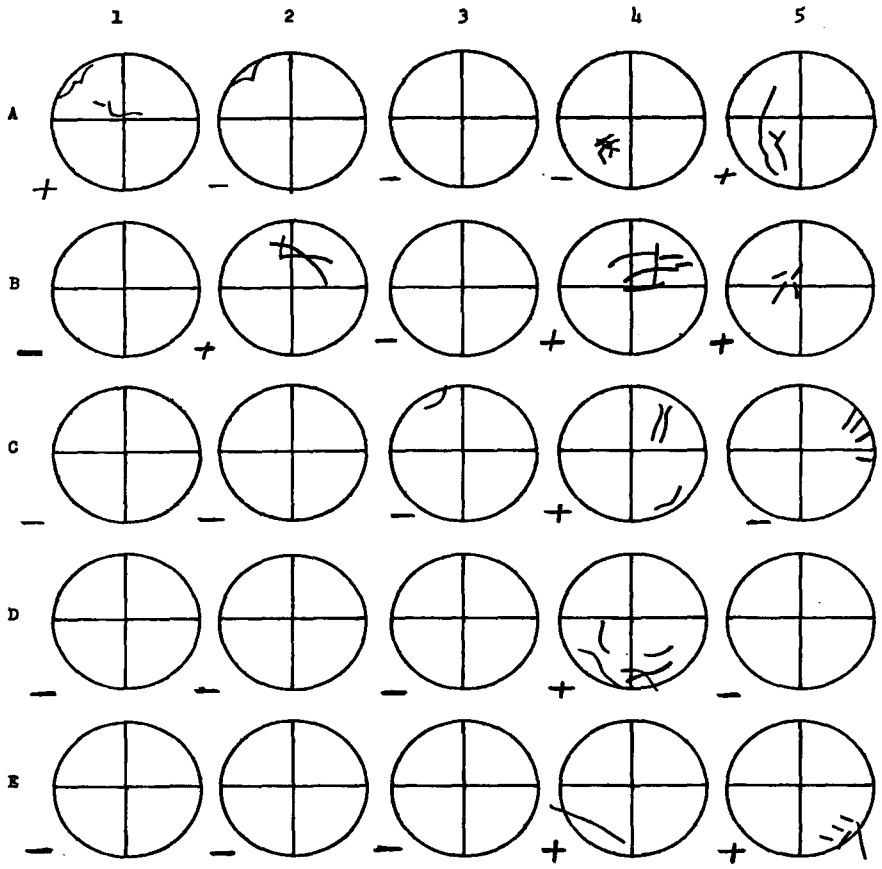

FIG. 2.—Permanent slide mold count record.

Slide No. 20; Positive Fields: 9; Analyst: LPW; Date: 3/4/52. *Directions.* Place the slide on the stage with the number at the right. The circles below correspond to the 25 fields on the permanent slide. In each circle, sketch all of the mold filaments seen in the corresponding field, indicating the relative size and position of each filament. Below each circle put (+) or (−) according to your findings.

placed on the pattern slide and pressed out to optimum thickness with a second cover slip. After thorough cooling, the excess sample is removed, the entire slide is cleaned, and the edges of the cover slips are sealed with fingernail polish. The layer of sample is quite tough, and the glycerine reduces the tendency to dry out. Some of our slides have been used for several years. Fig. 1 shows such a slide ready for examination.

A suitable report form is essential to the use of such permanent slides. This form consists of a letter-size sheet of paper on which 25 circles,

each about 1″ in diameter, are spaced. The mold filaments discovered in particular fields of the permanent slide are sketched in the corresponding circles on the report form. Such a form with the mold filaments found in the corresponding slide sketched in position is shown in Fig. 2. For each permanent slide we prepare a "master sheet" showing the filaments in each field. Using a blank report form, the analyst sketches in all of the supposed mold filaments that he finds in the fields of that slide. Comparison with the master sheet quickly shows whether the analyst has discovered all of the mold or whether he has classified as mold some filaments that do not meet the tests that have been established. All of the questionable filaments can again be examined with the help of the instructor who can emphasize to the student the appearance under the microscope of true mold filaments and focus the student's attention on the identification of the difficult filaments. The samples from which the slides are made are selected to cover all types of tomato products, all degrees of contamination, and especially those containing fine filaments that are difficult to find and identify.

It is interesting and encouraging to note how quickly the students learn to find most of the mold filaments and have difficulty only with those which are obscure or, for one reason or another, are difficult to identify. The student is impressed with the significance of the six tests which have been established for those filaments which are classified as mold in the official Howard mold count test (3). After examining a few such slides the analyst becomes confident that he can take any such permanent slide and find substantially all of the mold that is present. If he does not have such confidence at the end of the course, it is apparent that he has not obtained the necessary proficiency in the method, and must receive additional special training before he can make reliable counts. The proper use of such slides makes it possible for both the analyst and the instructor to know definitely the progress the student has been making.

Some of the analysts who are working for successive seasons find it helpful to come back to the school for a "refresher" period of instruction. The examination of a few permanent slides quickly establishes whether they have retained an appreciation of all of the details of mold counting.

SAMPLING DIFFICULTIES

As seen by the analysts, a tomato product is a mixture of tomato fibers and mold filaments. The difficulties of getting a proper sample of any such mixture are now recognized (7), and the necessity for taking all possible precautions to get a representative sample must be emphasized to all students. A complete explanation is not attempted, but in justice to the students, the limitations due to sampling difficulties are not

ignored. For instance, we do not infer that proper carrying out of the procedure will always result in exactly the same number of positive fields on each slide. We try to give a brief and simple explanation of the sampling difficulties so that the analyst will be reassured that the differences in positive fields on successive slides which he occasionally finds may well be due to the nature of the product he is examining and that he should not change in any way the method of carrying on the tests or identifying the filaments (8). We are aware of the psychological hazard of instinctively expecting about the same number of positive fields on successive slides. We emphasize that the method must be carried out exactly in every detail, and the positive fields recorded as they are found on the slides. Consideration of the results must be left until the examination is finished. If the first two slides do not agree within a reasonable number (plus or minus 2 or 3 positive fields) an additional slide or more should be counted and all results should be included in calculating the final count.

INTERPRETATION OF THE MOLD COUNT

The Howard mold count has been carried out in essentially the same manner since it was originated in 1912. Through the years a large amount of data and experience have been accumulated with respect to the significance of the mold count in terms of the character of the raw product and the methods used in the washing and sorting operations (8–12). The validity of the Howard mold count method has been upheld in numerous court trials. So long as the interpretation of results rests on this foundation, we feel strongly that any change in the method should be in the direction of greater uniformity and reproducibility of results and ease of operation, and should not affect the magnitude of the results. Our whole endeavor is to teach the method exactly as it is given in *Official Methods of Analysis*. Only those counts made in accordance with the exact official procedure can be interpreted by the manufacturer or by regulatory agencies.

SELECTION OF THE FIELDS IN MOLD COUNTING

Experienced analysts find that it is advantageous to have some mechanical means of selecting in an objective manner those fields that are to be counted on the regular Howard mold count slide. Several methods of doing so are available. A diagram may be fastened to the stage of the microscope so that a particular point of the mechanical stage or of the slide itself can be used as an indicator in going from one field to the next (13). Some operators learn that a particular angular turn of the knobs of the mechanical stage will move the slide the desired distance. Probably the most successful means is to make a "pattern cover slip."

A cellophane pattern with 25 holes, each about 1.40 millimeters in diameter, is fixed in Canada balsam to the top of a Howard mold count cover slip under a regular microscope cover slip. This pattern cover slip is used in the regular way and, if handled carefully, it will last for a long time. Each of the holes is slightly larger than the field delineated by proper setting of the microscope itself, but the location of each field to be examined is indicated by the holes in the pattern. Several experienced analysts have expressed the thought that the manufacturers of the Howard mold count slide should make permanent markings on one side of the cover slip or on the disc of the slide to designate these fields. Thus far the manufacturers have not considered it worth while to do so.

INSECT FRAGMENTS IN COMMINUTED FOOD PRODUCTS

In certain collaborative studies of comminuted vegetable and fruit products experienced analysts repeatedly reported gross differences in the number of insect fragments. The details of the procedure were therefore studied in three stages. First, the identification of the fragments was studied through the preparation of permanent slides containing difficult fragments of known history. By means of the "pattern slide" described above, one fragment was cemented in each of the areas designated by the circles. They were embedded in Canada balsam and covered with a regular cover slip. The fragments could be examined from the top and the bottom, but of course could not be moved with the needle. At first there were differences in the ability of experienced analysts to identify the fragments. After two or three such permanent slides had been examined, however, the analysts were able to identify at least 85 per cent of the fragments. (Each of the slides had some fragments of vegetable origin along with those of animal origin.) These slides were sent with the master sheets from one laboratory to another in different parts of the country, and they seem to have established the ability of the analysts to identify the fragments.

There was still uncertainty as to the efficiency of the extraction procedure in removing the fragments for identification. Even though consistent results were obtained on duplicate samples, it was not known whether all of the fragments present or only a consistent proportion of them were recovered. This portion of the method was examined by the use of test fragments. Satisfactory test fragments were prepared from the outer skin of a larva, such as the corn ear worm. This outer skin is thin and tough and has characteristic spines. The skin was cut into strips, each about 0.2 mm wide, with a microtome. Each strip was then cut with a scalpel into small parallelograms on the stage of a Greenough microscope. Known numbers of these fragments were put directly into each analytical subdivision. Since each of the test fragments had four straight

sides, it was considered highly improbable that any naturally occurring fragment would be confused with the test fragments. Since all of the variables of sampling were thus eliminated, it was possible to study the efficiency of the extraction procedure by means of the recovery of the test fragments. Such samples were sent to a large number of analysts in different sections of the country. Gross differences in recovery were obtained. Some analysts recovered every one of the test fragments; other analysts recovered only 15 or 20 per cent. Correspondence did not reveal the differences in procedure which resulted in such wide variation in recovery of fragments.

Finally, the instructor of the group which had obtained uniformly high counts gave personal instruction to the other analysts in the details of the extraction procedure. It was found that variations in the details of technic, all within the broad language of the method itself, resulted in the differences in recovery. The following are some of the types of details which were found to be important: (1) having the inside of the glass chemically clean before each determination; (2) using a larger amount of water in transferring the samples, so that the sample and water total at least 600 ml in volume before the extracting oil is added; (3) mixing the oil with the sample by a gentle plunging action, so that no air is drawn into the mixture and the oil particles are made small but not fine enough to result in an emulsion; and (4) thorough washing of the neck of the flask with hot water, etc. By following all of these details, each of the analysts was able to get at least 80 per cent recovery of the fragments.

Although we do not have any way of verifying the assumption, it seems probable that those details that result in a high recovery of test fragments will likewise result in the separation and identification of a similar proportion of the naturally occurring fragments. At any rate, by following the details of the procedure, all analysts now seem to be able to get similar results. It is gratifying to find that by having the steps of this procedure written down in detail it is now possible for other experienced analysts to get satisfactory results without receiving personal instruction in the method.

The testing of finely comminuted cereals presents special difficulties. The treatment of the sample before extraction, and especially the discovery and identification of such fine fragments, requires special personal instruction.

FLY EGGS IN TOMATO PRODUCTS

The A.O.A.C. methods appear to be satisfactory. Using known numbers of house-fly eggs, we have obtained good recovery. We find that coloring the cloth filter with a blue dye makes the eggs easier to see.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Washington, D. C., 1950.
(2) *Microanalysis of Food and Drug Products*, Food and Drug Circular No. 1, pp. 160–161.
(3) SMITH, H. R., *The Canning Trade*, March 29, 1948.
(4) BIGELOW, W. D., *This Journal*, **3**, 453 (1917–1920).
(5) WILDMAN, J. D., *Science*, **77**, 263 (1933).
(6) SMITH, H. R., *Ind. Eng. Chem.*, **7**, 286 (1935).
(7) WILDMAN, J. D., *This Journal*, **14**, 563 (1931).
(8) SMITH, H. R., *The Canning Trade*, July 5, 1948.
(9) HOWARD, B. J., and STEPHENSON, C. H., *The Sanitary Control of Tomato Canning Factories*, U. S. Department of Agriculture Bulletin 569, June, 1917.
(10) ———, *Microscopical Studies on Tomato Products*, U. S. Department of Agriculture, Bulletin 581, October, 1917.
(11) EISENBERG, W. V., *Western Canner and Packer*, **44**, No. 15, April, 1952.
(12) SMITH, H. R., *The Canning Trade*, **74**, 29 (1952).
(13) BEACH, P. L., *Food Engineering*, October, 1951, p. 112.

THE IDENTIFICATION OF DUNG AND PLANT FRAGMENTS IN DAIRY PRODUCTS*

By DOROTHY B. SCOTT (Division of Microbiology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

The determintion of dung and plant fragments in dairy products is based essentially on a knowledge of the histology of plant tissues in the feed of ruminant animals and in dung fragments. A comparison of the tissues before and after digestion in the ruminant animals can be made microscopically.

In previous reports by Duggan (1) and by Scott (2), staining technics were given as aids to identification. Discrimination was based on the staining of nutritive elements in the plant tissues, which were removed by digestion. The undigested fragments generally did not take the stain. The presence of mucilage, which remained unstained on the surface of dung fragments, was also used as an important characteristic (3).

Botanists have used ruthenium red (ammoniacal ruthenium oxychloride, a colored mineral salt) as a biological stain for pectic substances since it was introduced by Mangin in 1888 (4). Since there is a degradation of pectic substances in the animal's digestive system, the use of ruthenium red as a stain for plant fragments before and after digestion was investigated.

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Pectic substances occur in the tissues of higher plants in the intercellular layer or middle lamella, and in the primary wall of meristematic and parenchymous tissue cells (5). They are derivatives of polygalacturonic acid and occur in three general types: protopectin, pectin, and pectic acid.

Protopectin is insoluble in water and is the parent substance which yields pectin upon hydrolysis. Water-soluble pectin is a group name for all pectic substances between protopectin and pectic acid (6). Pectic acid may be obtained by the hydrolysis of pectin.

Pectic substances occur as deposits on cellulose (5). The bond is weak.

The early belief that the cementing pectic substance in the middle lamella was chiefly calcium pectate has been supported by the later studies of Joslyn and Phaff (5). They state that the evidence is definitely in support of the theory that the pectic substances in the intercellular layers occur as insoluble calcium polygalacturonates, and that magnesium may also be present.

In 1930, Tetley (7) made a microchemical study of the cell walls of apple tissue and concluded that ruthenium red was not specific for all pectic substances. She found that protopectin and pectin did not take the stain, whereas pectic acid and pectates did. When pectin was made slightly alkaline with sodium or potassium hydroxide, the resulting jelly was stained.

Kerr and Bailey (8) used ruthenium red as a stain for a large number of organic compounds in the cambium and its derivative tissues. The compounds which they found would take the stain were: (*a*) coagulated protoplasm and nuclei; (*b*) certain lipoids; and (*c*) polysaccharides that contain glucuronic acids or galacturonic acid, that is, pectic compounds such as gums, mucilages, hemi-celluloses, oxycellulose, etc.

An extensive study was made in 1936 by Bonner (9), who also stated that ruthenium red was not specific for pectins but was specific for the sixth carboxyl group. He found that cellulose which was mildly oxidized and other "plant slimes" which contain such carboxyl groups were also stained by ruthenium red.

In recent studies of cotton fiber morphology (10), ruthenium red was found to stain the primary wall a deep pink, whereas the secondary wall remained unstained. Therefore, in the plant tissues of feed, the cell walls which contain some pectic substances together with some other nutritive substances may be expected to stain a deep pink.

Degradation of the pectic substances is caused by enzymes. The three recognized as most important (11) are: (*a*) *protopectinase* which converts the protopectin of the cell wall into soluble pectin, and also attacks the cementing intercellular substances; (*b*) *pectinases* which hydrolyze soluble pectin, pectic acid, or pectates; and (*c*) *pectases* or *pectinesterases* which

attack only soluble pectin by splitting off methyl alcohol to form pectinic and pectic acids. The pectic enzymes occur in various plant tissues and microorganisms, specifically the fungi and bacteria (12).

Werch, Jung, Day, and Friedman (13) found that pectin was decomposed in the digestive system of humans and dogs with a recovery of 10 per cent or less in the feces. They reported that the degradation of pectin was caused by the bacterial enzymes of the colon. The most active organisms belonged to the groups: *Aerobacillus*, *Lactobacillus*, *Micrococcus*, and *Enterococcus*.

Kertesz (14) states that pectin added experimentally to the diet of animals and human subjects cannot be recovered in the feces. The pectin passes through the esophagus, stomach, and small intestines without decomposition, but is subsequently decomposed by the enzymes produced by microorganisms.

In a previous report (15), the effect of the digestive processes of ruminant animals on plant tissues has been discussed. The meristematic tissues and, generally, the primary tissues are digested. The proteinaceous substances and other nutritive elements that might stain with ruthenium red will also be utilized for nourishment by the ruminants.

The lignified cellulose of the secondary tissue, which comprises most of the undigested plant fragments found in dung, will not stain with ruthenium red.

Combinations of compounds with cellulose other than lignin, such as fats and waxes (of which cork is an example (6)) will not stain with ruthenium red. The walls of the outer cells of the stems of grasses contain high percentages of silica (16). These fragments in dung do not stain with ruthenium red.

Loose spirals with secondary walls of the vascular elements are frequently found in dung. Jagged ends of dung fragments may be caused by the degradation of the pectic substances by the digestive enzymes.

PROCEDURE

(1) Immerse fragments in 5 ml of 1.0% $CaCl_2$ soln for 5 min. in a fritted glass funnel.

(2) Drain and wash thoroly with H_2O.

(3) Add 5 ml of 1% chrome alum soln and drain after 5 min.

(4) Wash thoroly with H_2O.

(5) Add 5 ml of ruthenium red soln (2 mg of ammoniated ruthenium oxychloride dissolved in 5 ml of H_2O. Add an equal vol. of 95% ethyl alcohol and mix).

(6) Stain for 15 min., then drain.

(7) Wash with 10 ml of 1+1 95% alcohol, then with H_2O.

(8) Add 5 ml 0.5% soln of chrome alum and drain immediately.

(9) Wash with 5 ml 1% acetic acid soln, then with H_2O.

(10) Transfer with H_2O to filter paper and examine with the stereoscopic microscope at 60–70×.

NOTES

Filter all solutions before using. Precipitates may mask the mucilage.

Ruthenium red in solution decomposes readily; thus a fresh solution must be made up each time.

Calcium chloride solution helps to keep the intercellular substance insoluble.

Chrome alum is used both as a mordant and for de-staining.

The degradation of the insoluble pectinates in the middle lamella can be demonstrated by treating the fragments with a dilute solution of Calgon before staining. Unadjusted Calgon (sodium polymetaphosphate) is a calcium sequestering agent. In dilute solution, it is slightly alkaline and its affinity for calcium changes the calcium pectinate to a pectic substance which allows the intercellular substance remaining in the fragments to take the stain.

Plant fragments found in dairy products have been subjected to varying conditions of chemical treatment, microorganisms, and physical effects so that they may differ in physical microscopic characteristics from normal plant tissues.

In developing a staining technic with a dilute ruthenium red solution, it was necessary to work out an empirical procedure to emphasize the portions of the tissue that take the ruthenium red stain and those that remain unstained.

The cellulose associated with lignin and with waxy and fatty substances, as well as with chloroplasts and other compounds resistant to enzymic action, do not stain deeply with ruthenium red. There may be a slight pink tinge.

The staining technic developed will stain the primary cell walls and some nutritive elements in plant fragments a deep pink. The dung fragments which consist mostly of cellulose combined with lignin, minerals, and waxy and fatty substances either do not stain at all or show only a slight pink tinge. The mucilage on the surface of the dung fragments does not generally stain deeply, if at all.

SUMMARY

(1) Ruthenium red in dilute solutions will stain cell walls which contain some pectic substances a deep pink. Some nutritive elements may also stain. In general, when the special technic for staining plant tissues with ruthenium red is used, the pectic substances and nutritive elements which take the stain are those that are digested in the intestinal tract of ruminant animals.

(2) The mucilage on the surface of dung fragments does not take the stain in most cases.

(3) Dung fragments in which there is not complete digestion of pectic substances in the primary walls and some nutritive elements that pass the ruminant's digestive system may take the stain in part.

REFERENCES

(1) DUGGAN, R. E., *This Journal*, **28**, 331 (1944).
(2) SCOTT, D. B., *ibid.*, **36**, 301 (1953).
(3) ———, *ibid.*, **34**, 350 (1951).
(4) MANGIN, L., *Compt. rend.*, 1888–1893 Series.
(5) JOSLYN, M. A., and PHAFF, H. J., *Wallerstein Lab. Communs.*, **10**, 29 (1947).

(6) *Matthew's Textile Fibers*, 5th Ed., John Wiley and Sons, Inc., New York, 1947, p. 100.
(7) TETLEY, U., *J. Pomol. Hort. Sci.*, **8**, 153 (1930).
(8) KERR, T., and BAILEY, I., *J. Arnold Arboretum*, **15**, 327 (1934).
(9) BONNER, J., *Botan. Rev.*, **2**, 475 (1936).
(10) MOORE, A. T., *Stain Technology*, **28**, 149 (1953).
(11) PHAFF, H. J., and JOSELYN, M. A., *Wallerstein Lab. Communs.*, **10**, 30 (1947).
(12) FOSTER, J. W., *Chemistry of the Fungi*, Academic Press, Inc., New York, 1949, p. 546.
(13) WERCH, S. C., JUNG, R. W., DAY, A. A., and FRIEDMAN, T. E., *J. Infectious Diseases*, **70**, 231 (1942).
(14) KERTESZ, Z. I., *J. Nutrition*, **20**, 289 (1940).
(15) SCOTT, D. B., *This Journal*, **35**, 330 (1952).
(16) EAMES, A. J., and MACDANIELS, L. H., *An Introduction to Plant Anatomy*, McGraw-Hill Book Company, New York, 1947.

(This concludes the symposium on extraneous materials in foods and drugs. —Ed.)

MICROBIOLOGICAL PROCEDURE FOR THE ASSAY OF PANTOTHENIC ACID IN FOODS: RESULTS COMPARED WITH THOSE BY BIOASSAY*

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Liberation of pantothenic acid from biological materials by the use of intestinal phosphatase-pigeon liver enzymes (as developed by Novelli, *et al.* (1), for the liberation of microbiologically active pantothenic acid from coenzyme A) has been shown by Neilands and Strong (2) to be greater than liberation with the previously used mylase-P digestion. Bioassays conducted by Lih, *et al.* (3), in which rat growth was used, confirm the biological activity of pantothenic acid in pantothenic acid conjugates. For concentrates high in bound pantothenic acid, these workers obtained relatively good agreement between the microbiological assay following the "phosphatase-liver enzyme treatment" and the rat growth assay. However, for natural products such as liver, yeast, wheat bran, and rice bran, they found that the bioassay gave higher values.

The relatively high amount of pantothenic acid in the blank from the pigeon liver enzyme extract limited the use of phosphatase-liver treatment to high potency materials. To overcome this difficulty, Novelli and Schmetz (4) treated the liver extract with Dowex activated with *N* HCl. This removed most of the pantothenic acid from the enzyme solution, reduced the blank without destroying the specific enzyme activity required, and thus made it possible to assay materials low in pantothenic acid.

This paper reports results of studies to develop, standardize, and evaluate procedures for determining free and total pantothenic acid in foods through application of the findings cited above.

PROCEDURES

Microbiological assay procedure for free and total pantothenic acid.—The test organism was *Lactobacillus arabinosus* 17-5 held on yeast agar, twice

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transferred through liver-fortified basal medium, and twice washed with sterile physiological salt solution before use as inoculum. The basal medium was that of Skeggs and Wright (5), modified to include double xanthine and 10 mg of Tween "80" per 100 ml of double strength medium. Preparation of solutions and handling of ingredients for the basal medium were the same as those given in Agriculture Handbook 29 (6). Assays were carried out with fifteen levels of standard and five levels of each sample in triplicate tubes, incubated at 34°C. for 72 hours and titrated with standard 0.1 *N* NaOH, with bromthymol blue as indicator.

Intestinal phosphatase (Armour) as received was stored in a freezer. The pigeon liver powder, also obtained from Armour, was first sifted through a 50-mesh screen to remove extraneous material and stored in 10 gram lots in the freezer.

For use in assay, 2 per cent intestinal phosphatase was prepared as needed by shaking vigorously until all the solid was in solution. The heavy foam was allowed to settle for at least half an hour in the refrigerator, and the solution was used without further treatment.

By using the freezing compartment of a refrigerator to chill and re-chill thoroughly all equipment between steps, and by restricting the time of centrifugation to 5 minutes at 3000 r.p.m., equipment usually found in laboratories was easily adapted for the pigeon liver extraction and purification. Ten grams of the pigeon liver powder was extracted with 100 ml of 0.02 *N* potassium bicarbonate (ice cold) by rubbing it in a mortar held in an ice-salt bath; the solids were then removed by centrifuging. One hundred g of Dowex-1 (200–400 mesh, L-2993-12) activated with *N* HCl was prepared essentially as described by Novelli and Schmetz (4), except that stirring for 10 minutes was substituted for each wash and filtering with suction was substituted for centrifugation. One-half of the activated Dowex was mixed with the potassium bicarbonate extract of the pigeon liver powder and centrifuged; the supernatant liquid was decanted, mixed with the remaining half of the Dowex, and then centrifuged again. Further treatments with Dowex were found to be of no practical advantage. The purified liver enzyme preparations were stored frozen in sterile plugged tubes in the freezing compartment of the refrigerator, and each tube was thawed just prior to use. Judging from the results obtained with the standard yeast sample no loss of activity was apparent after a month's storage under these conditions.

Finely ground weighed samples of foods of sufficient size (determined by preliminary tests) to give about 0.01 mmg pantothenic acid per ml of test solution were blended with 10 ml *M* "tris" buffer of *p*H 8.3, tris (hydroxymethyl) aminomethane, [2-amino-2-(hydroxymethyl)-1,3-propanediol; P-4883 Eastman], and water; autoclaved 15 minutes at 15 pounds steam pressure with caprylic alcohol used to prevent foaming; cooled, and made to a volume of 200 ml. If the suspension of sample was

not sufficiently stable to permit withdrawal of a representative aliquot, the mixture was re-blended briefly just prior to aliquoting.

To determine the free pantothenic acid value, aliquots of the food extracts were treated (omitting enzymes) under conditions identical to those described below. Since the free pantothenic acid was always less than the total, the aliquots taken for determination were usually twice those used for enzyme treatment.

To determine the total pantothenic acid value, the following "phosphatase-liver enzyme" treatment was carried out prior to the microbiological assay of the food. For the enzyme incubation, the aliquot of samples should not exceed 1.4 ml; the usual aliquots should be 0.4, 0.6, and 0.8 ml. The aliquots of the samples were placed in tubes held in an ice bath; 0.1 ml of 0.1 *M* sodium bicarbonate buffer, 0.4 ml of 2 per cent intestinal phosphatase, 0.2 ml of the just-thawed 10 per cent pigeon liver solution (twice treated with Dowex) and water were added to make a final volume of 2.1 ml per tube. Blanks were run with each assay with levels of 1, 1½, and 2 times the amount of the liver enzyme solution described above for use with the food samples. A sample of brewer's yeast,[1] especially prepared for use as a standard, was tested each time unknown food samples were assayed. All tubes were layered with toluene, plugged with cotton, and incubated 4 hours at 37°C. or overnight, if more convenient. After incubation, the mixtures were made to a volume of 100 ml and filtered through Whatman No. 40 fluted filter paper. The filtrates were used directly in 1, 2, 3, 4, and 5 ml amounts in the assay tubes. Calculations were made from the titrations of the standard alkali plotted against concentrations of pantothenic acid (0.01–0.1 mmg) on log-log paper to obtain the standard curve.

Bioassay procedure for total pantothenic acid.—In a rat growth assay of the standard brewer's yeast, conducted at Beltsville, weanling male rats were fed a commercial diet deficient in pantothenic acid (averaging 0.6 mmg/g total pantothenic acid as determined by the microbiological procedures described above) for 2 weeks. Litter mates were then divided into five groups of 12 each. One group continued to receive the pantothenic acid-deficient diet only; the second and third groups received diet supplements of 35 mmg and 140 mmg calcium pantothenate three times a week respectively; the fourth and fifth groups received 290 mg and 1100 mg of the standard yeast three times a week, respectively.

Later, rat growth tests, in which a slightly different procedure was used, were conducted on five common foods (carrots, egg powder, kale, peanuts, and pork liver) at College Station, Texas. Twelve rats, 6 males and 6 females chosen at random were fed at each level of supplementation, after an average of 21 days on a pantothenic acid-deficient diet (Texas No. 969). The calcium pantothenate standard at three levels (10, 20, and

[1] Obtained fresh from Christian Heurich Brewing Co., Washington, D. C.

40 mmg) and each food at two levels were given daily. Food levels were chosen on the basis of preliminary microbiological assays and calculated to give the equivalent of 10–40 mmg calcium pantothenate. The supplements given ranged from 75 to 1200 mg daily. The calculations were made according to Bliss (6–8, 9) and varied very little from those obtained by plotting average weekly gains against daily supplements on semi-logarithmic coordinates.

RESULTS

Phosphatase-liver enzyme activity tests.—The studies of the ability of intestinal phosphatase and of pigeon liver enzyme to release pantothenic acid from natural conjugates and make it available to the microorganism used for assay, included use of (*a*) no enzymes; (*b*) increasing amounts of intestinal phosphatase with a constant amount of pigeon liver enzyme; and (*c*) increasing amounts of pigeon liver enzyme with a constant amount of intestinal phosphatase with 6, 9, and 12 mg aliquots of the standard yeast sample. Results are given in Table 1. These data show that in a material high in bound and total pantothenic acid such as yeast, a minimum of 0.2 ml of 2 per cent intestinal phosphatase and 0.1 ml of the 10 per cent twice-Dowex-treated pigeon liver enzyme are needed for the maximum release. Twice these amounts were adopted for use in the

TABLE 1.—*Release of bound pantothenic acid in a brewer's yeast sample by varying levels of intestinal phosphatase and pigeon liver enzymes*

NO. OF SAMPLES	INTESTINAL PHOSPHATASE, 2 PER CENT	PIGEON LIVER ENZYME PREPARATION, 10 PER CENT	TOTAL PANTOTHENIC ACID FOUND[a]	BOUND PANTOTHENIC ACID RELEASED[a]
	ml	*ml*	*per cent*	*per cent*
9	0	0	45	0
9	0	0.1	54	16
2	0.025	0.1	85	72
3	0.05	0.1	82	68
6	0.1	0.1	82	67
3	0.2	0.1	100	100
15	0.4	0.1	100	100
3	0.6	0.1	94	89
9	0.4	0	74	52
6	0.4	0.01	78	59
3	0.4	0.025	81	66
3	0.4	0.05	88	78
15	0.4	0.1	100	100
3	0.4	0.2	100	99
3	0.4	0.3	94	88
6	0.4	0.4	102	103

[a] As per cent of the value obtained with 0.4 ml phosphatase and 0.1 ml pigeon liver.

TABLE 2.—*Pantothenic acid values obtained by microbiological assays using either intestinal phosphatase and pigeon liver, mylase-P, or no enzymes*

FOOD	PHOSPHATASE AND LIVER		DIFFERENCE BETWEEN MEANS	MYLASE-P		DIFFERENCE BETWEEN MEANS	NO ENZYME	
	NO. OF DETNS	MEAN		NO. OF DETNS	MEAN		NO. OF DETNS	MEAN
		mmg/g			*mmg/g*			*mmg/g*
Beef, lean round	11	23.4±0.89[a]	5.8[b]	7	17.6±0.64[a]	8.9[b]	6	8.7±0.21[a]
Carrots	15	20.7±0.55	5.9[b]	6	14.8±0.43	0.6	9	14.2±0.19
Whole egg powder	9	76.2±1.70	10.3[b]	7	65.9±1.37	15.2[b]	6	50.7±1.69
Kale	15	64.9±1.63	17.4[b]	10	47.5±1.30	6.1[b]	9	41.4±1.11
Peanuts	9	23.8±0.75	8.4[b]	5	15.4±0.66	3.0[b]	6	12.4±0.37
Liver, pork	16	203.0±5.00	61.0[b]	16	142.0±7.52	46.0[b]	6	96.0±1.14
Spinach	7	21.6±1.96	6.1[b]	7	15.5±0.18	6.2[b]	4	9.3±0.39
Whole wheat	12	11.0±0.41	3.4[b]	6	7.6±0.13	1.7[b]	6	5.9±0.22
Yeast, brewer's	42	117.6±2.21	58.8[b]	16	58.8±2.64	12.5[b]	42	46.3±0.52
Yeast, Pabst	41	89.3±4.38	32.6[b]	8	56.8±4.08	48.8[b]	39	8.0±0.13

[a] Standard error of the mean.
[b] Significant at 1% level.

standardized procedure. Other studies with yeast, in which first one enzyme was used and then the other, or both together, showed that the simultaneous action was best. Experiments demonstrated that mylase-P added to the samples along with, before, or after the other two enzymes did not further increase the amount of pantothenic acid found.

The standardized phosphatase-liver enzyme treatment was then compared directly with mylase-P and with no enzyme treatment prior to microbiological assay. Tests were made on ten foods or food products: a commercial preparation of whole egg powder, Pabst yeast, and eight foods obtained fresh (the edible portion was separated and air dried over calcium chloride where necessary, and ground and mixed to give a representative sample).

Mylase-P was used in 10 times the usual amounts or gram for gram in these samples. As shown in Table 2, the pantothenic acid values obtained by using the standardized procedure with phosphatase-liver enzymes were significantly greater for all samples than the values found with mylase-P. With the exception of carrots, which showed no difference, the use of mylase-P gave significantly greater amounts of pantothenic acid than the use of no enzymes.

Reproducibility of phosphatase-liver enzyme treatments.—To test further the ratio of enzyme concentration to sample weight and to find the reproducibility of results with different sized samples for extraction, an-

TABLE 3.—*Pantothenic acid values of non-fat milk solids with increasing sample size*

WT. OF SAMPLE ETXD PER 200 ML	NO. OF SUBSAMPLES	WT. OF SAMPLE ALIQUOT FOR ENZYME TREATMENT[a]	AV. TOTAL PANTOTHENIC ACID	
			PER G SAMPLE	PER CENT OF MEAN[b]
g		*mg*	*mmg/g*	*per cent*
10	3	20	35.0	107
12.5	3	25	32.5	99
10	3	30	34.7	106
15	3	30	32.0	98
18	3	36	31.0	95
12.5	3	38	30.5	93
10	3	40	32.7	100
15	3	45	32.6	99
12.5	3	50	30.0	91
18	3	54	34.4	105
15	2	60	32.6	99
18	3	72	33.5	102
50	1	105	35.8	109
50	1	160	34.4	105
50	1	210	34.7	106

[a] Enzyme concentration held at 0.4 ml 2% intestinal phosphatase and 0.2 ml 10% Dowex treated pigeon liver. Total volume for enzyme incubation: 2.1 ml/16 hr incubation at 37°C.
[b] Mean of 38 determinations = 32.8 mmg/g.

TABLE 4.—*Pantothenic acid values obtained by rat bioassay and by microbiological assays using intestinal phosphatase-pigeon liver enzymes or mylase-P*

FOOD	BIOASSAY (RAT)			MICROBIOLOGICAL ASSAY			
				PHOSPHATASE AND LIVER		MYLASE- P	
	NO. OF ANIMALS	MEAN	95% LIMITS	MEAN	95% LIMITS	MEAN	95% LIMITS
		mmg/g	*mmg/g*	*mmg/g*	*mmg/g*	*mmg/g*	*mmg/g*
Carrots	60	35.7	51.4– 24.9	20.7[b]	21.9– 19.5	14.8[c]	15.9– 13.7
Whole egg powder	60	61.5	83.0– 45.6	76.2	80.1– 72.3	65.9	69.3– 62.6
Kale	48[a]	56.2	81.7– 38.6	64.9	68.4– 61.4	47.5	50.4– 44.6
Peanuts	60	25.4	33.6– 19.3	23.8	25.5– 22.1	15.4[b]	17.2– 13.6
Liver, pork	60	176.7	244.7–127.5	203.0	213.7–192.3	142.0	158.0–126.0
Yeast, brewer's	48	138.0	169.0–112.7	117.6	122.1–113.1	58.8[c]	64.4– 53.1

[a] High level supplement not eaten; therefore only low level supplement animals used.
[b] Significant difference from bioassay mean.
[c] Highly significant difference from bioassay mean.

other series of tests were made on three subsamples of a composite of commercially prepared non-fat milk solids. The data shown in Table 3 represented a five-fold variation in sample size for extraction and a ten-fold change in sample size for enzyme incubation. The mean of the 38 determinations was 32.8 mmg pantothenic acid per gram. The total pantothenic acid found ranged irregularly from 91 to 109 per cent of the mean value, showing that there was no relationship between the sample sizes taken for extraction or the sizes of the aliquots used for enzyme incubation.

Bioassays.—Values obtained by rat bioassay on six widely different foods are given in Table 4, together with the values obtained by both microbiological procedures. The negative controls in the Beltsville test on brewer's yeast averaged 4.3 and those in the College Station tests averaged 5.0 grams gain per week.

The results by the microbiological assay, with phosphatase-liver enzymes used for incubation of the samples, agreed with the results by bioassay with the exception of carrots. There is a possibility that carrots may contain some unknown enzyme inhibitors or a growth-stimulating factor for rats not included in the basal diet. When compared with the bioassay values the lower values obtained by microbiological assay with mylase-P showed highly significant differences for carrots and brewer's yeast and a significant difference for peanuts.

The 95 per cent limits of the bioassay values were calculated from the equations of Bliss and the limits of the microbiological assays were calculated from the standard errors of the means given in Table 2.

DISCUSSION AND CONCLUSIONS

A procedure for the microbiological assay of foods for total pantothenic acid content using the phosphatase-liver enzymes for preparation of sample extracts has been adapted for use with ordinary laboratory equipment. Excellent reproducibility of the standardized procedure was obtained over a wide range of sample sizes. In the microbiological assay of ten foods, the standard error averaged 3.7 per cent of the means. The pantothenic acid values on foods were statistically greater in all cases than those obtained with mylase-P. Rat bioassays of five of these foods gave values in good agreement with those obtained by the standardized microbiological procedure. It has been proved possible to obtain reasonably reliable values for the total pantothenic acid content of foods by the standardized microbiological procedure with the use of *L. arabinosus* and phosphatase-liver treatment of samples. It therefore seems justifiable to propose this method for use in tests on a much larger number of food samples and in a number of laboratories.

REFERENCES

(1) NOVELLI, G. D., KAPLAN, N. O., and LIPMANN, F., *J. Biol. Chem.*, **177**, 97 (1949).
(2) NEILANDS, J. B., and STRONG, F. M., *Arch. Biochem.*, **19**, 287 (1948).
(3) LIH, H., KING, T. E., HIGGINS, H., BAUMANN, C. A., and STRONG, F. M., *J. Nutrition*, **44**, 361 (1951).
(4) NOVELLI, G. D., and SCHMETZ, F. J., JR., *J. Biol. Chem.*, **192**, 181 (1951).
(5) SKEGGS, H. R., and WRIGHT, L. D., *ibid.*, **156**, 21 (1944).
(6) TOEPFER, E. W., ZOOK, E. G., ORR, M. L., and RICHARDSON, L. R., U. S. Department of Agriculture, Agriculture Handbook No. 29 (1951).
(7) GORMI, G., *Proc. Soc. Exptl. Biol. Med.*, **62**, 1251 (1946).
(8) BLISS, C. I., *Biometrics Bull.*, **1**, 57 (1945).
(9) GYÖRGY, P., *Vitamin Methods*, Vol. 2, Academic Press, Inc., New York, 1951.

DETERMINATION OF DICHLOROPHENE, HEXACHLOROPHENE, AND 2,2'-THIOBIS(4,6-DICHLOROPHENOL) IN SOAP AND COSMETIC PREPARATIONS*

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The methods available for the determination of dichlorophene, hexachlorophene, and 2,2'-thiobis(4,6-dichlorophenol)† in soap or cosmetic preparations are non-specific. They are, for the most part, colorimetric methods in which a color is obtained by reaction of the phenolic group with a suitable reagent (1, 2).

Obvious advantages are offered by a method which makes possible both the identification and the quantitative determination of these compounds. The following spectrophotometric method has therefore been devised. Any one of the three compounds can be separated from most of the interfering substances, and can subsequently be identified and determined by ultraviolet spectrophotometry.

METHOD

APPARATUS

The apparatus consists of a spectrophotometer capable of isolating a wave band of 5 mμ, or less, in the spectral region 250–360 mμ. A Cary Recording Spectrophotometer, Model 11, was used.

REAGENTS

Standard dichlorophene, hexachlorophene, and 2,2'-thiobis(4,6-dichlorophenol) solutions (50 mg/l in alcohol).—Dissolve 100 mg of the compound in 100 ml of

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† Sold under the name "Actamer" by Monsanto Chemical Co., St. Louis, Mo.

alcohol. Dilute a 10 ml aliquot of this soln to exactly 200 ml with alcohol. [The dichlorophene, hexachlorophene, and 2,2′-thiobis(4,6-dichlorophenol) used in this investigation were white crystalline compounds melting at 177.5–178.5°C., 165°C., and 188.0–188.5°C., respectively.]

Alcoholic NaOH soln.—Dissolve 1 g of solid NaOH in ca 100 ml of 25% alcohol.

Isopropyl ether.—Wash 200 ml of a good commercial grade of isopropyl ether once with 50 ml of ca 0.1 *N* NaOH. Follow this with two 50 ml water washings.

Methanol soln, 80%.—Dilute 80 ml of absolute methanol to 100 ml with H_2O.

Petroleum ether.—B. P. 30–60°C.

PREPARATION OF SAMPLE

Soaps.—Weigh accurately, in a 250 ml beaker, 0.5–1.0 g soap shavings. Add 50 ml H_2O and heat mixt. on a hot plate with constant stirring until soap dissolves. Transfer soln to a 250 ml separatory funnel, cool to room temp., and proceed as directed under DETERMINATION.

Creams and pastes.—Weigh accurately in a weighing bottle a sample estimated to contain 5–10 mg of the ingredient sought. Add 10 ml H_2O to sample, stir into thin paste, and transfer paste to a 250 ml beaker with a little alcohol. Add 75 ml of alcohol, heat soln to boiling, and then cool to 10–15°C. in an ice bath. Filter soln thru quant. filter paper into a 400 ml beaker. Reserve filtrate. Return filter paper and residue to original beaker, add 10 ml of H_2O, and macerate filter paper thoroly. Repeat extn with another 75 ml of alcohol and filter into previously reserved filtrate. Wash filter paper with a little alcohol, and then discard filter paper and residue. Evap. soln (ca 200 ml) to ca 30 ml on the steam bath under a gentle jet of air. Proceed as directed under DETERMINATION.

DETERMINATION

(a) *When sulfated surface active agent is present.*—Transfer prepd. soln to a 250 ml Erlenmeyer flask, add 10 ml of concd HCl, attach flask to a water-cooled condenser, and reflux soln for 15 min. Cool soln to room temp. transfer to a 250 ml separatory funnel, and proceed as directed under **(b)**, beginning with "ext. with three 30 ml portions of $CHCl_3$ . . . ."

(b) *When sulfated surface active agent is absent.*—Transfer prepd. soln to a 250 ml separatory funnel with 20 ml of H_2O, acidify with concd HCl and ext. with three 30 ml portions of $CHCl_3$. Filter combined $CHCl_3$ exts. thru a cotton plug into a 250 ml beaker and wash plug with a little $CHCl_3$. Evap. $CHCl_3$ on a steam bath under a gentle jet of air, and dissolve residue in 30 ml of hot petr. ether.

Transfer the resulting soln to a 100 ml separatory funnel with 20 ml of petr. ether and ext. with four 30 ml portions of 80% methanol. Place combined methanol exts. in a 500 ml separatory funnel, add 300 ml of H_2O, 1 ml of concd HCl, and ext. with three 30 ml portions of isopropyl ether. Filter ether exts. thru cotton plug into a 250 ml beaker and wash plug with a little ether. Evap. ether soln to ca 20 ml on the steam bath under a gentle jet of air, transfer soln to a 100 ml separatory funnel with 10 ml of isopropyl ether and ext. with five 25 ml portions of alcoholic NaOH soln. Discard isopropyl ether.

Transfer combined alk. ext. to a 250 ml separatory funnel, acidify with concd HCl and ext. with three 30 ml portions of ethyl ether. Filter combined ether exts. thru a cotton plug into a 250 ml beaker, and wash plug with ca 30 ml of ether. Evap. ether on the steam bath under a gentle jet of air.

Dissolve residue in alcohol, transfer to a 100 ml volumetric flask and make to

vol. with alcohol. (A suitable absorbancy reading can be obtained with this diln if the sample contains 5–10 mg of dichlorophene, hexachlorophene, or 2,2′-thiobis(4,-6-dichlorophenol). If the reading is too high, make a further diln.)

For dichlorophene, det. absorbancy of standard and sample solns at 260, 288, and 316 mμ. Calculate per cent dichlorophene in the sample as follows:

Per cent dichlorophene

$$= \frac{C_s}{100} \times \frac{A_{\text{sample 288 m}\mu} - \frac{(A_{\text{sample 260 m}\mu} + A_{\text{sample 316 m}\mu})}{2}}{A_{\text{std 288 m}\mu} - \frac{(A_{\text{std 260 m}\mu} + A_{\text{std 316 m}\mu})}{2}} \times \frac{1}{\text{Wt of sample (g)}}$$

For hexachlorophene, det. absorbancy of standard and sample solns at 270, 300, and 330 mμ. Calc. per cent hexachlorophene in sample:

Per cent hexachlorophene

$$= \frac{C_s}{100} \times \frac{A_{\text{sample 300 m}\mu} - \frac{(A_{\text{sample 270 m}\mu} + A_{\text{sample 330 m}\mu})}{2}}{A_{\text{std 300 m}\mu} - \frac{(A_{\text{std 270 m}\mu} + A_{\text{std 330 m}\mu})}{2}} \times \frac{1}{\text{Wt of sample (g)}}$$

For 2,2′-thiobis(4,6-dichlorophenol), det. absorbancy of standard and sample soln at 262, 308, and 354 mμ. Calc per cent 2,2′-thiobis(4,6-dichlorophenol) in sample:

Per cent 2,2′-thiobis(4,6-dichlorophenol)

$$= \frac{C_s}{100} \times \frac{A_{\text{sample 308 m}\mu} - \frac{(A_{\text{sample 262 m}\mu} + A_{\text{sample 354 m}\mu})}{2}}{A_{\text{std 308 m}\mu} - \frac{(A_{\text{std 262 m}\mu} + A_{\text{std 354 m}\mu})}{2}} \times \frac{1}{\text{Wt of sample (g)}}$$

where C_s = concentration of standard in mg/l; A_{sample} = absorbancy of sample; and A_{std} = absorbancy of standard.

Det. absorbancy of sample soln at a sufficient number of points in the region 250–360 mμ to establish an absorbancy-wavelength curve.

EXPERIMENTAL

In Figs. 1, 2, and 3 are shown the ultraviolet absorption curves, respectively, of dichlorophene, hexachlorophene, and 2,2′-thiobis(4,6-dichlorophenol) in alcohol. Solutions of these compounds, varying in concentration from 20 to 100 mg/l, obey Beer's Law within ±1 per cent.

Samples of soap were prepared by dissolving 0.5 g portions of stearic acid in alcohol, and adding 0.1 *N* NaOH to bring the *p*H to about 10. Weighed amounts of dichlorophene, hexachlorophene, or 2,2′-thiobis(4,6-dichlorophenol) were then added, and the solutions were evaporated to dryness on the steam bath. Samples so prepared were then analyzed for dichlorophene, hexachlorophene, or 2,2′-thiobis(4,6-dichlorophenol) according to the proposed method. Recoveries are shown in Table 1. This method was then applied to four commercial brands of soap. The results are given in Tables 2 and 3.

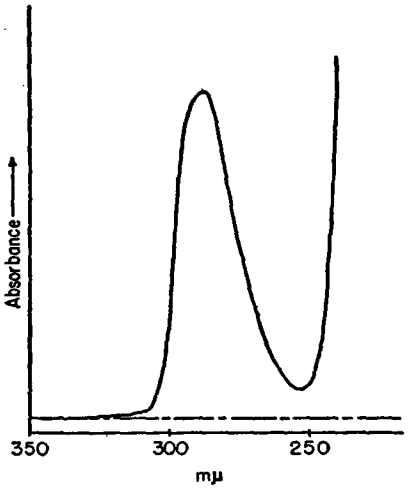


FIG. 1.—Absorption curve of dichlorophene (50 mg/l) in 95% EtOH.

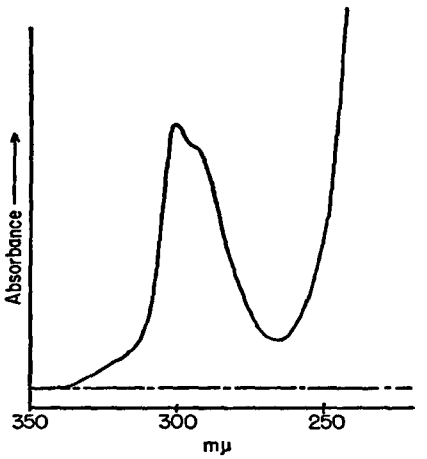


FIG. 2.—Absorption curve of hexachlorophene (50 mg/l) in 95% EtOH.

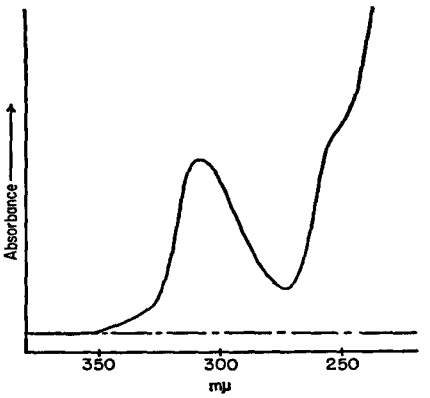


FIG. 3.—Absorption curve of 2,2′-thiobis(4,6-dichlorophenol), (25mg/l) in 95% EtOH.

A deodorant cream base, containing the following ingredients, was prepared:

Glyceryl monostearate	30 g
Stearic acid	6 g
Cetyl alcohol	6 g
Detyl Extra (isopropyl myristate-palmitate)	9 g

TABLE 1.—*Recoveries of dichlorophene, hexachlorophene, and 2,2'-thiobis (4,6-dichlorophenol) from soap*

SAMPLE COMPOSITION		RECOVERY	
SOAP	BACTERIOSTAT		
g	*mg*	*mg*	*per cent*
	Dichlorophene		
0.5	5	4.91	98.2
0.5	10	9.84	98.4
0.5	15	14.9	99.3
	Hexachlorophene		
0.5	5	4.82	96.4
0.5	10	9.52	95.2
0.5	15	14.5	96.7
	2,2'-Thiobis(4,6-dichlorophenol)		
0.5	5	4.91	98.2
0.5	10	9.97	99.7
0.5	15	14.9	99.3

TABLE 2.—*Recoveries of hexachlorophene from commercial brands of soap*

BRAND NO.	WT. OF SAMPLE	HEXACHLOROPHENE FOUND	
	g	*mg*	*per cent*
	0.5436	11.9	2.19
1	0.5066	10.8	2.13
	0.5030	10.7	2.13
	0.4583	9.80	2.14
2	0.5064	10.6	2.09
	0.4968	10.5	2.11
	0.4275	8.33	1.95
3	0.5183	8.98	1.73
	0.5057	9.73	1.92

TABLE 3.—*Recoveries of 2,2'-thiobis(4,6-dichlorophenol) from a commercial soap*

WEIGHT OF SAMPLE	2,2'-THIOBIS(4,6-DICHLOROPHENOL) FOUND	
g	*mg*	*per cent*
0.5422	10.6	1.95
0.5217	10.4	1.99
0.4710	9.10	1.93

Mineral oil	9 g
Titanium dioxide	12 g
Triethanolamine	1.5 g
Water	224.1 g

Upon analysis, no dichlorophene, hexachlorophene, or 2,2′-thiobis(4,6-dichlorophenol) was found in this cream base. Known amounts of di-

TABLE 4.—*Recoveries of dichlorophene, hexachlorophene, and 2,2′-thiobis (4,6-dichlorophenol) from deodorant cream base*

SAMPLE COMPOSITION		RECOVERY	
CREAM BASE	BACTERIOSTAT		
g	*mg*	*mg*	*per cent*
	Dichlorophene		
1.0	5	4.87	97.4
0.5	5	5.01	100.2
0.5	10	9.76	97.6
0.5	15	14.7	98.0
	Hexachlorophene		
1.0	5	4.93	98.6
0.5	5	4.73	94.6
0.5	10	9.32	93.2
0.5	15	14.7	98.0
	2,2′-Thiobis(4,6-Dichlorophenol)		
1.0	5	4.88	97.6
0.5	5	4.83	96.6
0.5	10	4.80	96.2

chlorophene, hexachlorophene, or 2,2′-thiobis(4,6-dichlorophenol) were then added to weighed samples of the cream base. Analyses were made by the proposed procedure. Recoveries are shown in Table 4.

The following cream containing a number of ingredients likely to be found in anti-perspirants was prepared:

Sodium lauryl sulfate	6 g
Glyceryl monostearate	18 g
Mineral oil	9 g
Spermaceti	15 g
Titanium dioxide	6 g
Glycerine	18 g
Aluminum sulfate	30 g
Urea	15 g
Water	161.5 g

Known amounts of dichlorophene, or 2,2′-thiobis(4,6-dichlorophenol) were added to portions of this cream and the resulting samples were analyzed. Recoveries are shown in Table 5.

DISCUSSION

So far as the authors are aware, dichlorophene has not been used in commercial soaps, and 2,2′-thiobis(4,6-dichlorophenol) has not been used in preparations other than soaps. Since it is possible that the uses of these

TABLE 5.—*Recoveries of dichlorophene and 2,2′-thiobis(4,6-dichlorophenol) from anti-perspirant cream*

COMPOSITION OF SAMPLE		RECOVERY	
ANTI-PERSPIRANT CREAM	BACTERIOSTAT		
g	*mg*	*mg*	*per cent*
	Dichlorophene		
1.0	5	4.83	96.6
1.0	5	4.90	98.0
	2,2′-Thiobis(4,6-Dichlorophenol)		
1.0	5	4.94	98.8
0.5	5	5.11	102.2
0.5	10	9.88	98.8
0.5	15	14.4	96.0

materials may be extended, the study has included the determination of dichlorophene, hexachlorophene, and 2,2′-thiobis(4,6-dichlorophenol) incorporated in various types of cosmetic preparations.

A small amount of material other than dichlorophene, hexachlorophene, or 2,2′-thiobis(4,6-dichlorophenol) is usually extracted in the procedure and interferes to a slight extent in the spectrophotometric determination. A "straight-line background" correction compensates for the presence of this material. In the equations given, the terms in parentheses are those needed for the correction.

Any other phenolic compounds present will interfere with the determination. Application of the variable reference technic of Jones, *et al.* (3), may be of value in obtaining satisfactory determinations when interfering substances are encountered. The complete ultraviolet curve of the unknown is plotted to establish the identity of the extracted material as dichlorophene, hexachlorophene, or 2,2′-thiobis(4,6-dichlorophenol).

NOTE

After the authors had completed this investigation, an article appeared in a British publication (4) describing a rapid ultraviolet spectrophotometric procedure for the detection and estimation of hexachlorophene in soap. The absorbancy measurements are made on a dilute KOH solution of the soap, and corrections are made for irrelevant absorbancy. The method is limited in scope because it depends on the complete solution of the cosmetic sample in a solvent. It is also probable that the background correction described will not be generally applicable.

REFERENCES

(1) GOTTLIEB, S., and MARSH, P. B., *Ind. Eng. Chem., Anal. Ed.*, **18**, 1, 16 (1946).
(2) LARSON, H. L., *J. Am. Oil Chemists Soc.*, **28**, 301 (1951).
(3) JONES, J. H., CLARK, G. R., and HARROW, L. S., *This Journal*, **34**, 135 (1951).
(4) LORD, J. W., MCADAM, I. A., and JONES, E. B., *Soaps, Perfumery, and Cosmetics*, **26**, 783 (1953).

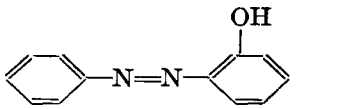
THE INFRARED SPECTRA OF SOME UNSULFONATED MONOAZO DYES

By MEYER DOLINSKY and JOHN H. JONES (Division of Cosmetics, Food and Drug Administration, Department of Health, Education and Welfare, Washington 25, D. C.)

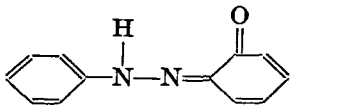
Monoazo dyes are commonly prepared by coupling a diazotized aromatic amine with an aromatic hydroxy or amino compound. Depending on the starting materials, the resulting azo dye may be an *ortho-* or *para*-hydroxyazo compound or an *ortho-* or *para*-aminoazo compound.

In the course of investigational work on the certifiable azo colors, the infrared spectra (in the region of 2 to 15 μ) of a number of unsulfonated azo dyes and related compounds were determined. The principal reason for obtaining these spectra was to provide a "working file" for use in identifying compounds which might be encountered in the work of this laboratory. A study of the curves, however, indicates that these data may also be of interest in connection with the problem of the structure of the compounds.

Although the structure of the azo dyes has been the subject of extensive investigation, considerable controversy still exists as to the exact nature of these compounds. The hydroxyazo compounds have been postulated as true hydroxy-azo compounds, as quinone-hydrazone compounds, and as mixtures of the two tautomeric forms (1–9).



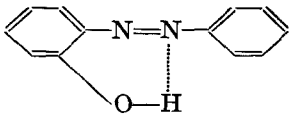
Hydroxyazo form



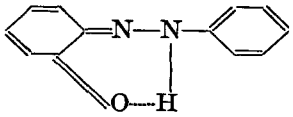
Quinone-hydrazone form

Evidence for the existence of these forms has been obtained from physical and chemical data or by comparison of the visible absorption spectrum with that of compounds of known structure.

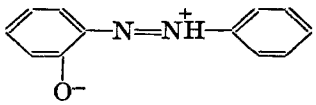
For the *o*-hydroxyazo compounds, some investigators have proposed a chelate type compound:



or



a zwitterion structure (10–16):



or a mesomeric form, as being more in accord with observed physical and chemical data.

EXPERIMENTAL

All spectra were obtained with a Perkin-Elmer infrared spectrophotometer, Model 21, equipped with rock-salt optics. The spectra were obtained for the solid compounds as films or mulls, and also, where possible, for solutions of the compounds in carbon disulfide. For those materials not sufficiently soluble in carbon disulfide, solution spectra in the 3 μ region were obtained in carbon tetrachloride with the use of 1 cm quartz cells. Typical spectra are shown in Figs. 1–31.

DISCUSSION

The infrared spectra of these compounds in the 3 μ and 6 μ region are of possible interest in connection with the various proposed structures for the hydroxyazo and aminoazo compounds. It has been shown previously

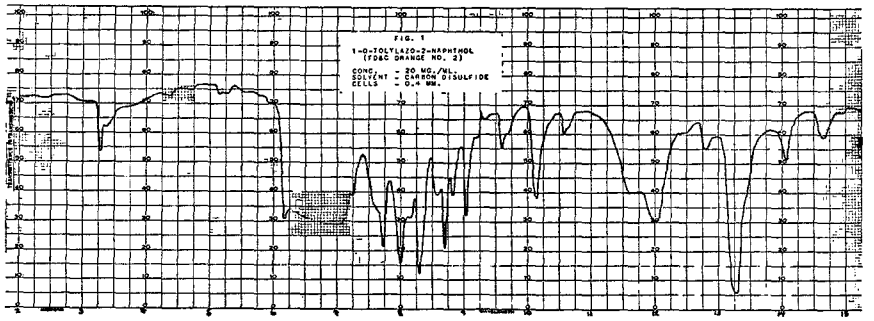


FIG. 1
1-O-TOLYLAZO-2-NAPHTHOL
(D&C ORANGE NO. 2)
CONC. - 20 MG./ML.
SOLVENT - CARBON DISULFIDE
CELLS - 0.4 MM.

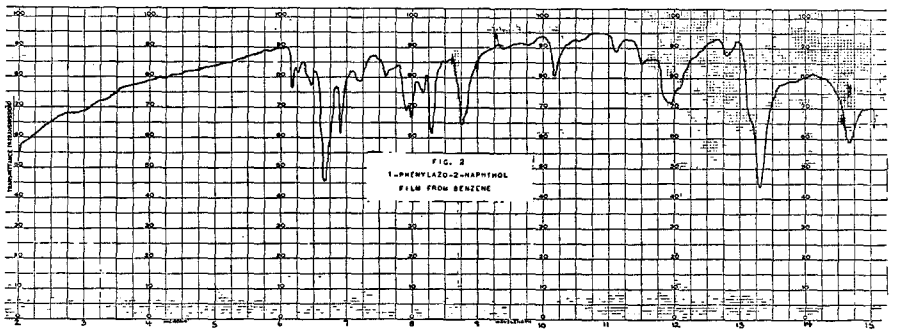


FIG. 2
1-PHENYLAZO-2-NAPHTHOL
FILM FROM BENZENE

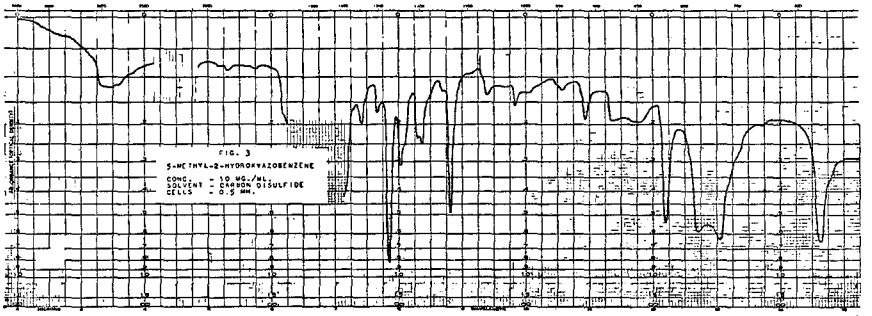


FIG. 3
5-METHYL-2-HYDROXYAZOBENZENE
CONC. - 10 MG./ML.
SOLVENT - CARBON DISULFIDE
CELLS - 0.5 MM.

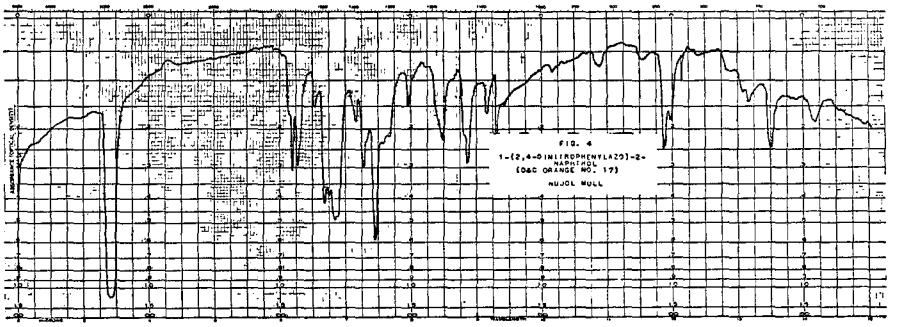


FIG. 4
1-(2,4-DINITROPHENYLAZO)-2-
NAPHTHOL
(D&C ORANGE NO. 17)
NUJOL MULL

FIGS. 1–4.

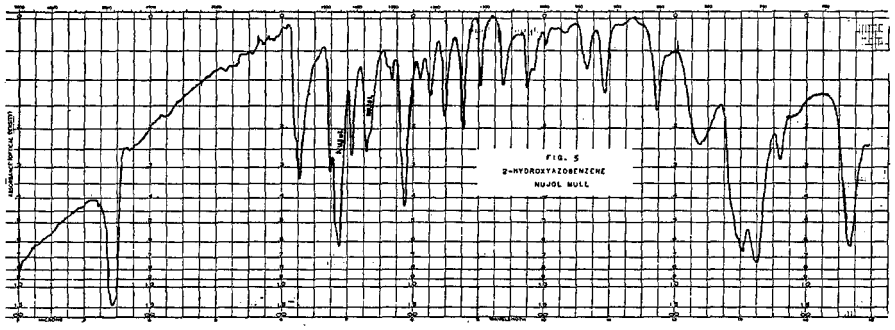


FIG. 5
2-HYDROXYAZOBENZENE
NUJOL MULL

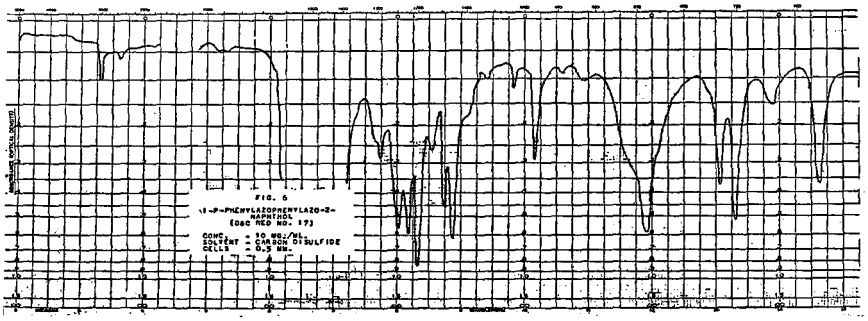


FIG. 6
1-P-PHENYLAZOPHENYLAZO-2-NAPHTHOL
(D&C RED NO. 17)
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

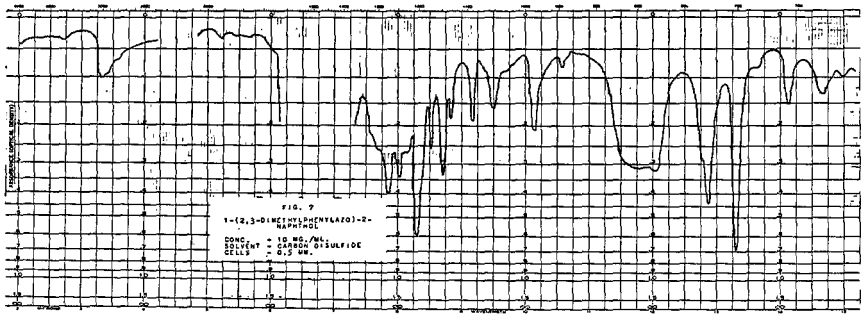


FIG. 7
1-(2,5-DIMETHYLPHENYLAZO)-2-NAPHTHOL
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

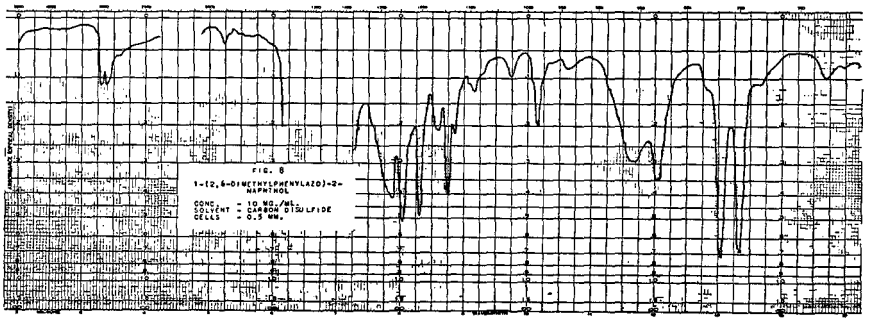


FIG. 8
1-(2,6-DIMETHYLPHENYLAZO)-2-NAPHTHOL
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

FIGS. 5–8

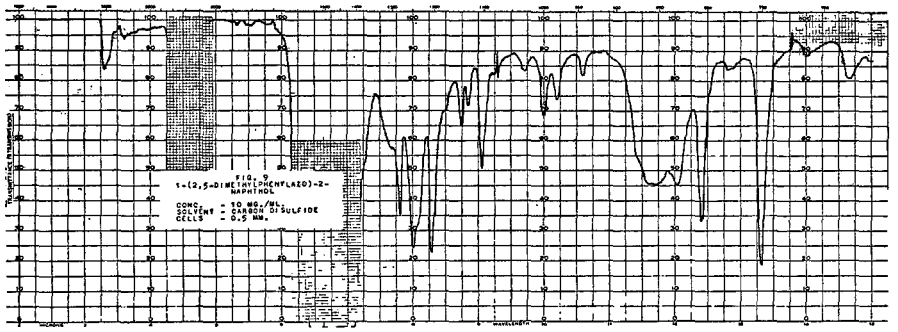


FIG. 9
1-(2,5-DIMETHYLPHENYLAZO)-2-NAPHTHOL
CONC. = 10 MG/ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

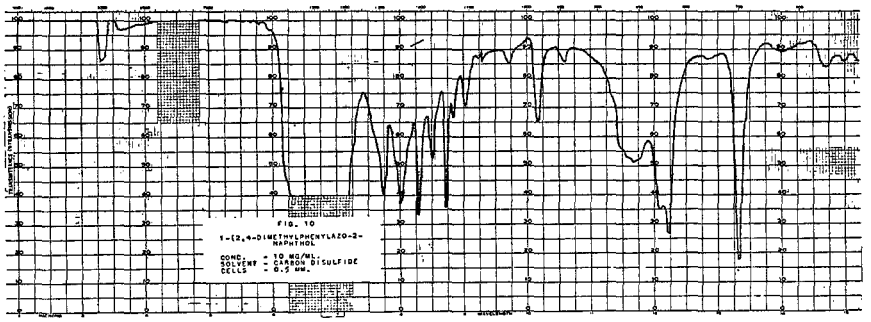


FIG. 10
1-(2,4-DIMETHYLPHENYLAZO-2-NAPHTHOL
CONC. = 10 MG/ML
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

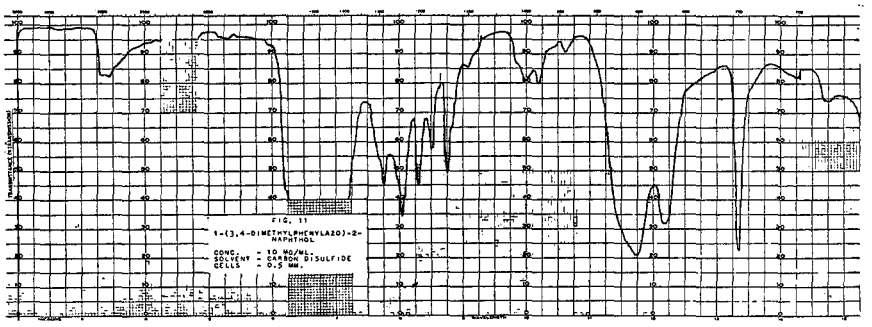


FIG. 11
1-(3,4-DIMETHYLPHENYLAZO)-2-NAPHTHOL
CONC. = 10 MG/ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

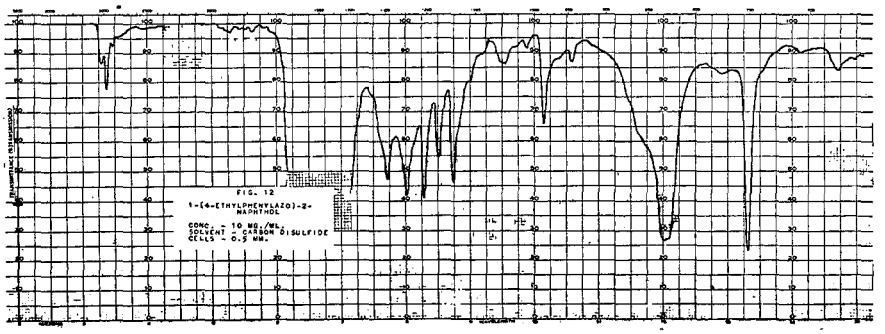


FIG. 12
1-(4-ETHYLPHENYLAZO)-2-NAPHTHOL
CONC. = 10 MG/ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

FIGS. 9–12

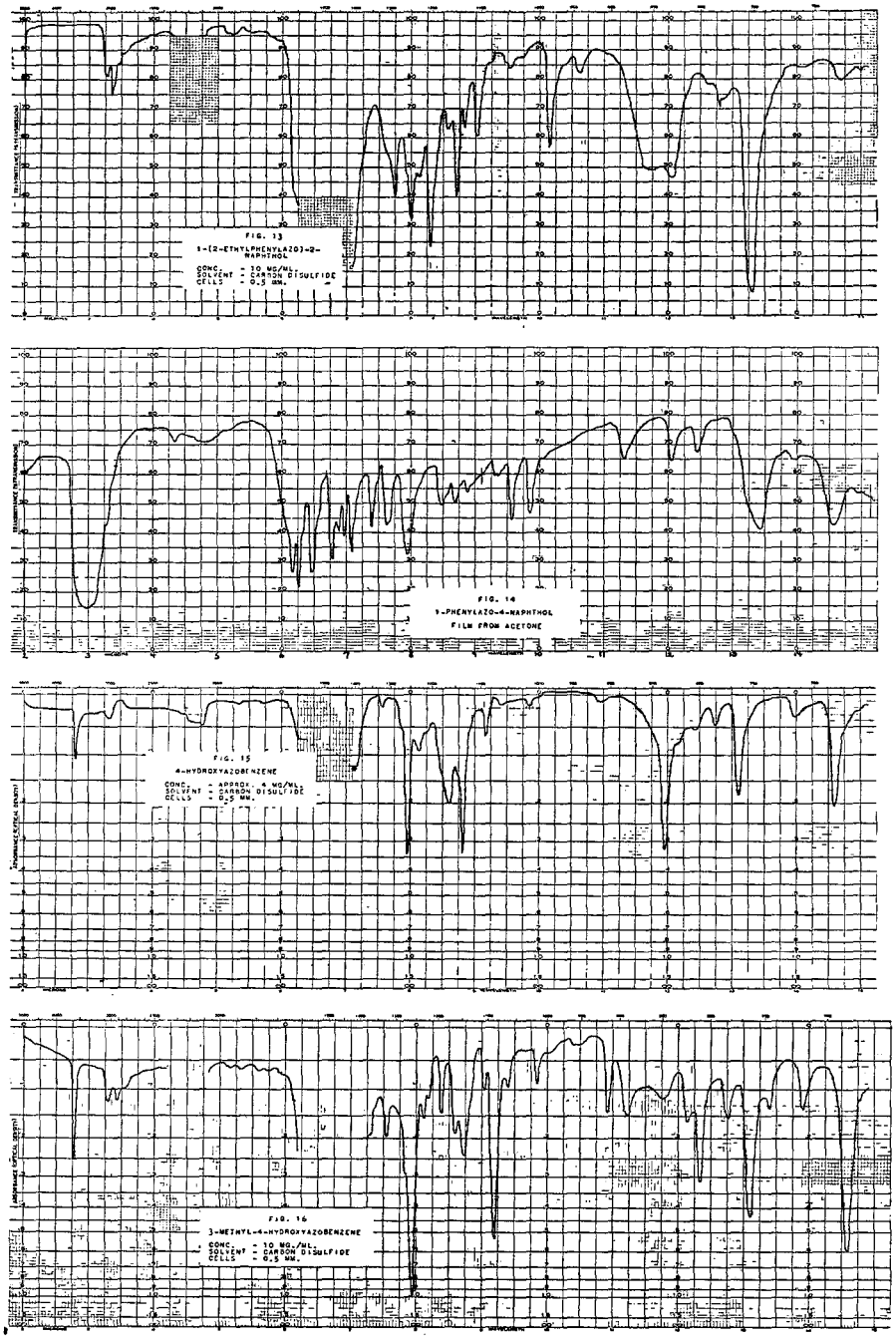


FIG. 13
1-(2-ETHYLPHENYLAZO)-2-NAPHTHOL
CONC. = 10 MG/ML
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.
FIG. 14
1-PHENYLAZO-4-NAPHTHOL
FILM FROM ACETONE
FIG. 15
4-HYDROXYAZOBENZENE
CONC. = APPROX. 4 MG/ML
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.
FIG. 16
3-METHYL-4-HYDROXYAZOBENZENE
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

FIGS. 13–16

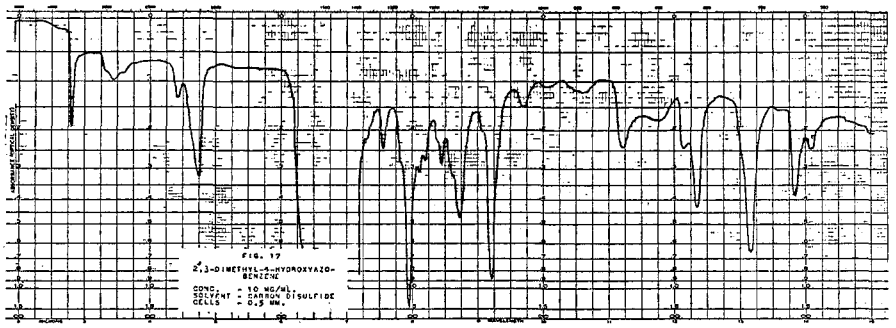


FIG. 17
2,3-DIMETHYL-4-HYDROXYAZO-BENZENE
CONC. = 10 MG/ML
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

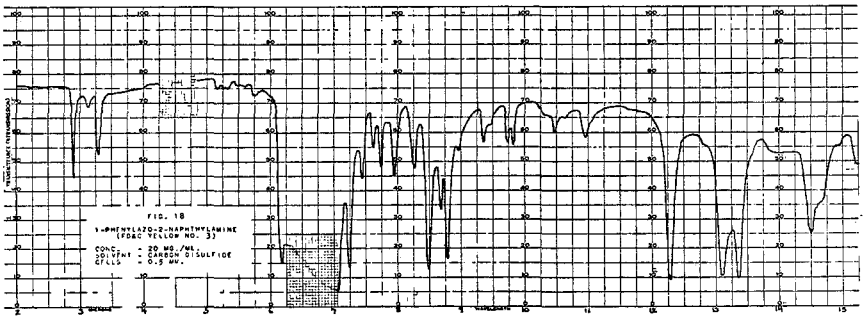


FIG. 18
1-PHENYLAZO-2-NAPHTHYLAMINE
(FDAC YELLOW NO. 3)
CONC. = 20 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

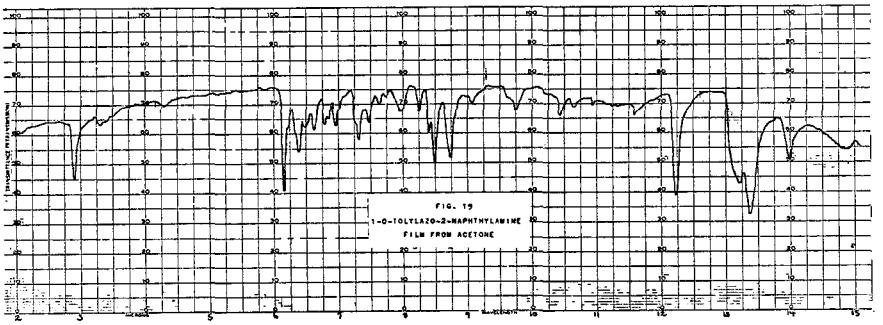


FIG. 19
1-O-TOLYLAZO-2-NAPHTHYLAMINE
FILM FROM ACETONE

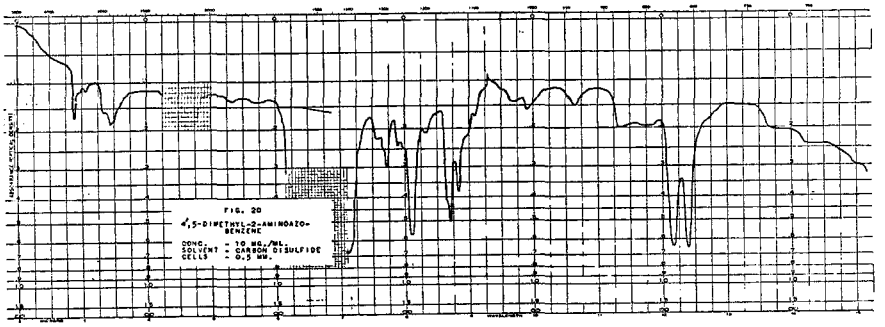


FIG. 20
4,5-DIMETHYL-2-AMINOAZO-BENZENE
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

FIGS. 17–20

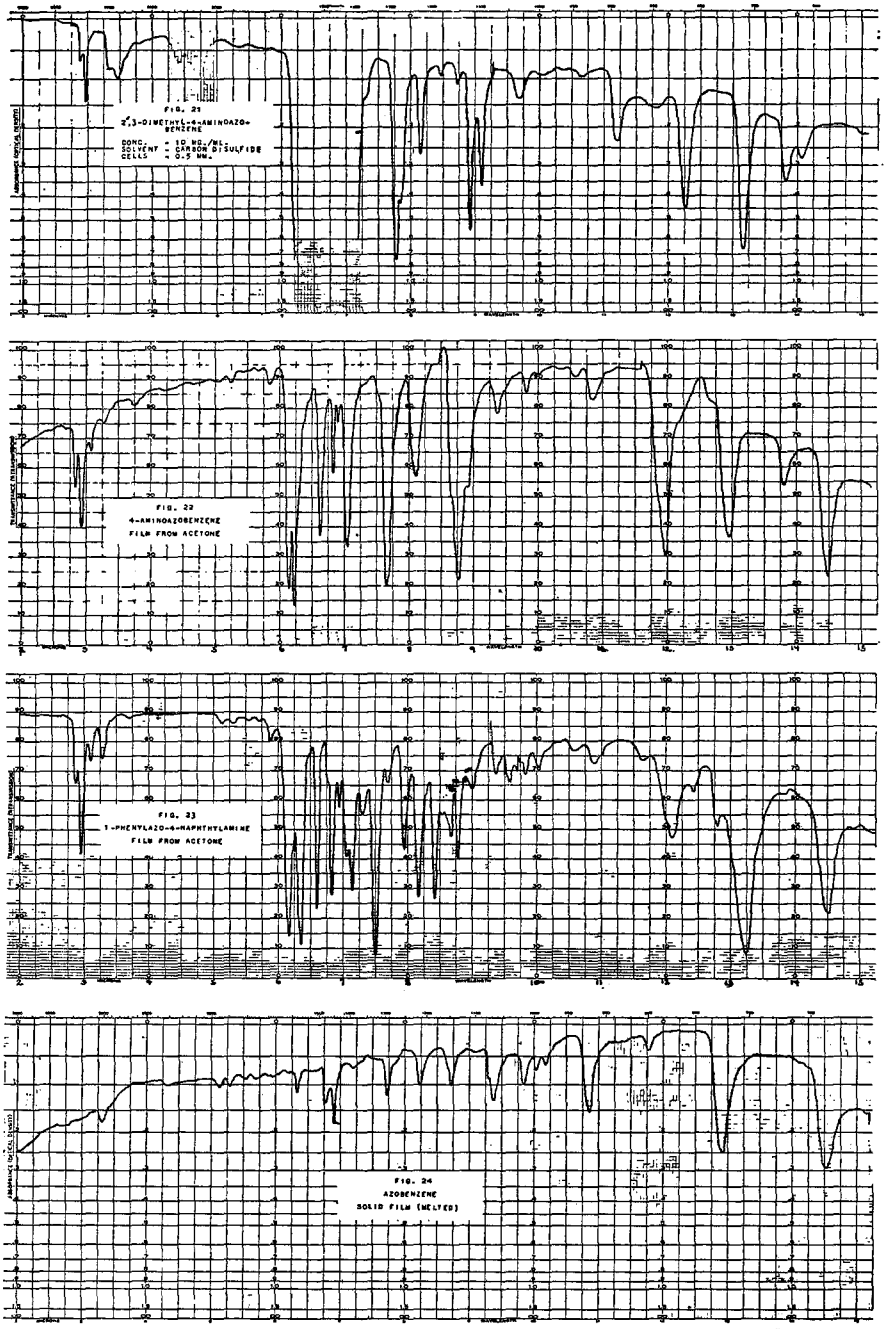


FIG. 21
2',3-DIMETHYL-4-AMINOAZO-BENZENE
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.
FIG. 22
4-AMINOAZOBENZENE
FILM FROM ACETONE
FIG. 23
1-PHENYLAZO-4-NAPHTHYLAMINE
FILM FROM ACETONE
FIG. 24
AZOBENZENE
SOLID FILM (MELTED)

FIGS. 21–24

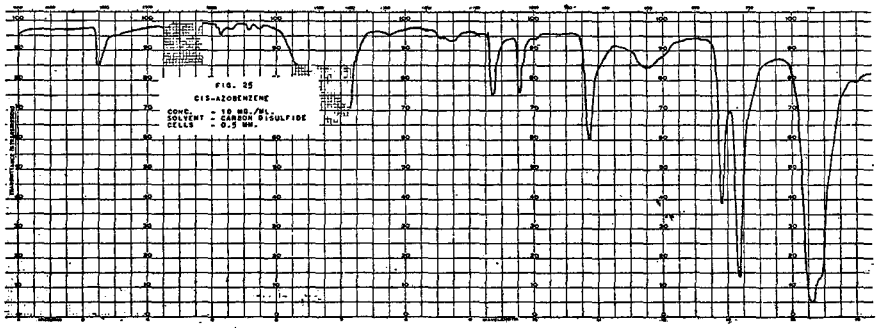


FIG. 25
CIS-AZOBENZENE
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

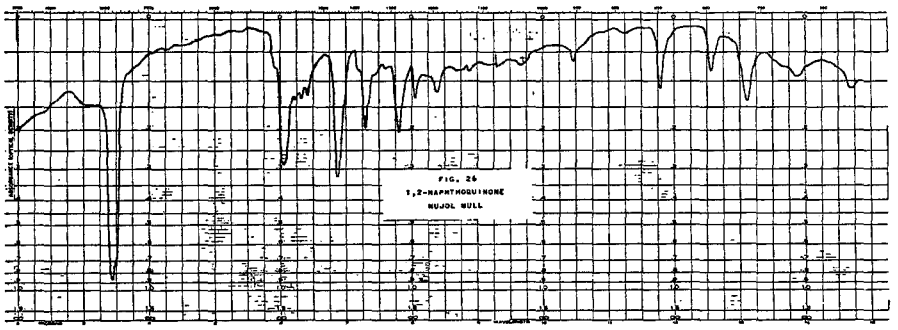


FIG. 26
1,2-NAPHTHOQUINONE
NUJOL MULL

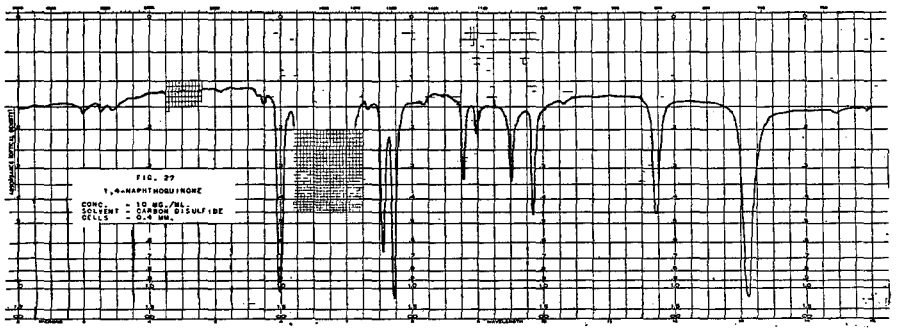


FIG. 27
1,4-NAPHTHOQUINONE
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.4 MM.

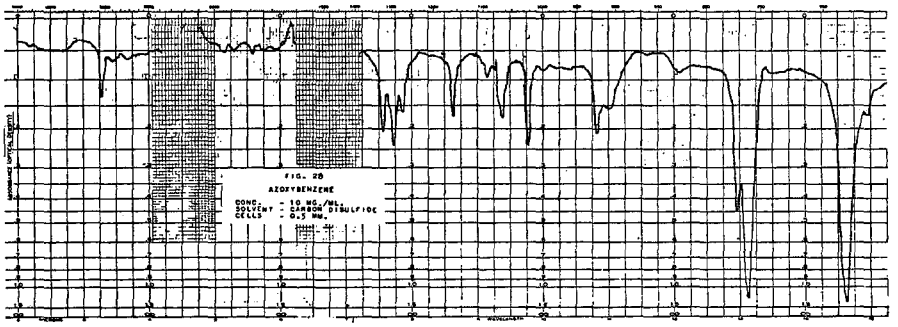
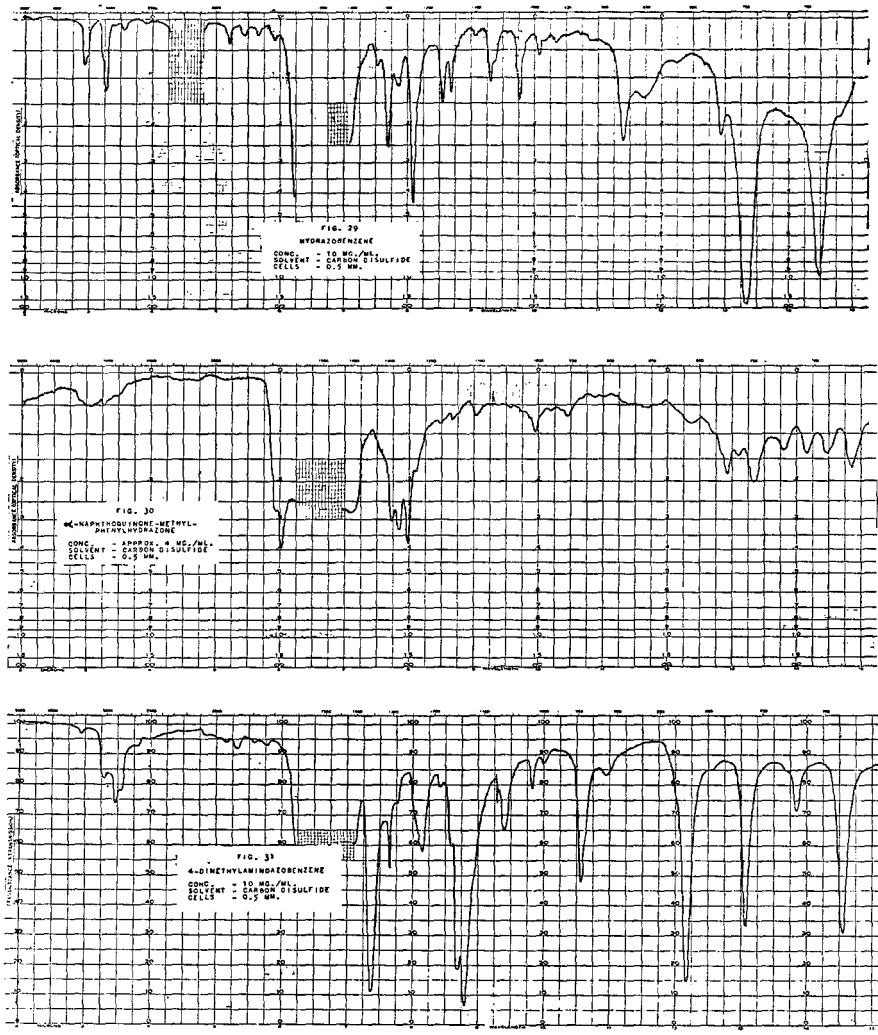


FIG. 28
AZOXYBENZENE
CONC. = 10 MG./ML.
SOLVENT = CARBON DISULFIDE
CELLS = 0.5 MM.

FIGS. 25–28



FIGS. 29–31

(17) that several *o*-hydroxyazo compounds, including phenylazo-β-naphthol and two of its derivatives as well as 2,2′-dihydroxyazobenzene, show no characteristic infrared absorption in the 3 μ region. On the other hand, 4-hydroxyazobenzene does show a distinct hydroxy absorption band at 2.8 μ. In the present study, no exceptions to this observation were found. None of the thirteen *o*-hydroxyazo compounds examined in the

present study showed any appreciable absorption in the 3 μ region which could be assigned to a hydroxy or NH group. All five of the *p*-hydroxyazo compounds showed distinct 3 μ hydroxy bands. No evidence of a characteristic "bonded" hydroxy absorption was noted in the 3 μ region for either *ortho* or *para* compounds.

Examination of the 6 μ region in the spectra of both *o*- and *p*-hydroxyazo compounds showed no peaks below 6.15 μ assignable to the carbonyl group. Compounds of known quinone structure, such as α- and β-naphthoquinone and α-naphthoquinone-methylphenylhydrazone, display strong 6 μ absorption bands. The absence of either hydroxy or carbonyl absorption in the spectra of the *o*-hydroxyazo compounds would appear to support the existence of a zwitterion type of structure for these substances, rather than a hydroxyazo-quinone-hydrazone tautomeric system.

The aminoazo compounds seem to have been studied less intensively than the hydroxyazo compounds. Presumably, their structure should be comparable to that of the corresponding *o*- and *p*-hydroxyazo compounds.

All the infrared spectra of the three *p*-aminoazo compounds examined show two distinct absorption peaks in the 3 μ region, indicating that these compounds must be primary amines. On the other hand, the three *o*-aminoazo compounds show only one strong absorption band in the 3 μ region; the second band is either very weak or absent. This may be considered as comparable with the *o*-hydroxyazo compounds, where the hydroxy absorption is completely lacking. Neither the *o*- or *p*-aminoazo compounds showed a characteristic absorption band in the 6 μ region assignable to an imine configuration.

Previous workers have indicated the presence of characteristic absorption due to the —N=N— group in the neighborhood of 7 μ (18, 19). Unfortunately, this absorption appears to be relatively weak in most azo dyes. Furthermore, it falls in a region where most aromatic compounds have relatively strong absorption bands. This azo absorption appears to be of little practical value as a general means of characterizing azo dyes.

Substituted benzene rings are known to show strong infrared absorption bands in the 11–15 μ region. The location of these bands is characteristic of the number and position of the ring substituents (20). These assignments appear to be valid for all of the azo dyes examined. For example, 4-hydroxyazobenzene shows absorption bands that indicate both monosubstituted and *para*-disubstituted benzene rings to be present.

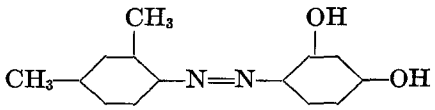
Examination of the infrared spectra of the unsulfonated monoazo dyes thus offers several points of information useful in characterizing these compounds. For example, the absence of hydroxy or amino absorption in the 3 μ region indicates an *o*-hydroxyazo compound; two distinct bands indicate a *p*-aminoazo compound; a combination of one strong and one weak band indicates an *o*-aminoazo compound; and a single 3 μ band indicates a *p*-hydroxy or possibly an *o*-aminoazo compound. The presence

of more than one amino or hydroxy group in the dye molecule would, of course, interfere with these assignments.

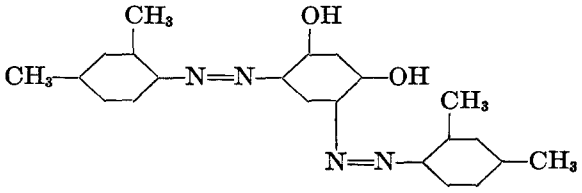
The characteristic infrared absorption in the "fingerprint region" offers a means by which any of the dyes may be uniquely characterized. For example, seven isomeric dyes obtained by coupling various diazotized xylidines and aminoethylbenzenes with 2-naphthol showed unique and easily recognized spectra. The identification of these isomeric dyes by other methods of analysis would be extremely difficult.

As a practical example of the use of the infrared spectra of these dyes, a subsidiary color of unknown structure was isolated from a sample of FD&C Yellow No. 4 (1-*o*-tolylazo-2-naphthylamine). This material was soluble in alkali and hence appeared to be a phenolic compound. Examination of the infrared spectrum of the unknown material showed the presence of a single strong absorption band in the 3 μ region, indicating a *para*-hydroxy group. From these data the compound was tentatively identified as *o*-tolylazo-*o*-cresol. The spectrum of a known sample of the latter was then obtained and found to be identical with that of the unknown.

In another case, a dye was submitted to the authors as *m*-xylene-azo resorcinol and was believed to have the following structure:



Examination of the infrared spectrum of the material showed the complete absence of a 3 μ absorption band, indicating that any OH groups present in the molecule must be *ortho* to an azo group, and hence that the structure assigned to the product could not be correct. Further examination of the dye showed it to be actually a diazo compound:



SUMMARY

The infrared spectra of a number of unsulfonated azo dyes and related compounds are given.

The absence of characteristic infrared peaks in the 3 μ and 6 μ regions appears to support the zwitterion type of structure for the *o*-hydroxyazo compounds, as opposed to a hydroxyazo-quinone-hydrazone tautomer.

The nature of the infrared absorption in the 3 μ and 11 to 15 μ regions

may be of value in establishing the location and nature of substituents in the azo dye molecule; furthermore, any of the unsulfonated monoazo dyes may be uniquely characterized by its infrared spectrum.

The characteristic band in the 7 μ region assignable to the azo group appears to be too weak to be of practical value in characterizing aromatic azo compounds.

REFERENCES

(1) BURAWOY, A., *Ann.*, **509**, 60 (1934).
(2) KUHN, R., and BÄR, F., *ibid.*, **516**, 143 (1935).
(3) AUWERS, K. V., and WOLTER, E., *ibid.*, **487**, 79 (1931).
(4) LAUER, W. M., and MILLER, S. E., *J. Am. Chem. Soc.*, **57**, 520 (1935).
(5) UEMURA, T., and INAMURA, Y., *Bull. Chem. Soc. Japan*, **10**, 169 (1953).
(6) BURAWOY, A., and MARKOWITSCH-BURAWOY, I., *Ann.*, **503**, 180–202 (1933); **504**, 71–80 (1933).
(7) BRADLEY, W., and ROBINSON, R., *J. Chem. Soc.*, **1934**, p. 1484.
(8) BORSCHE, W., MULLER, W., and BODENSTEIN, C., *Ann.*, **472**, 201–216 (1929).
(9) BURAWOY, A., SALEM, A. G., and THOMPSON, A. R., *J. Chem. Soc.*, 4793 (1932).
(10) MULLER, J. B., BLANGY, L., and FIERZ-DAVID, H. E., *Helv. Chim. Acta*, **33**, 2589 (1950).
(11) PFEIFFER, P., *J. Prakt. Chem.*, **126**, 108 (1930).
(12) MASON, F. A., *J. Soc. Dyers and Colourists*, **48**, 293 (1932)
(13) SLOTTA, K. H., and FRANKE, W., *Ber.*, **64**, 86 (1931).
(14) FIERZ-DAVID, H. E., BLANGY, L., and STREIT, H., *Helv. Chim. Acta*, **29**, 1718 (1946).
(15) ———, BLANGY, L., and MERIAN, E., *ibid.*, **34**, 846 (1951).
(16) SIDGWICK, N. V., "Organic Chemistry of Nitrogen," Oxford University Press, Oxford, England, 1942.
(17) HENDRICKS, S. B., WULF, O. R., HILBERT, G. E., and LIDDELL, U., *J. Am. Chem. Soc.*, **58**, 1995 (1936).
(18) WEST, W., and KILLINGSWORTH, R. B., *J. Chem. Phys.*, **6**, 1 (1938).
(19) LEFEVRE, R. J. W., O'DWYER, M. F., and WERNER, R. L., *Chem. & Ind.*, April 18, 1953.
(20) COLTHUP, N. B., "Spectra-Structure Correlations," Stamford Research Laboratories, American Cyanamid Company, Stamford, Conn.

DETERMINATION AND IDENTIFICATION OF ALLYL AND THIOBARBITURATES IN THE PRESENCE OF OTHER BARBITURATES

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The hypnotic and sedative drugs on the market have been mixed so that a wide variety of combinations is offered for sale. The assay of each preparation, for the most part, presents an individual problem. In some preparations, two barbiturates have been mixed to give a desired physiological effect. Barbiturates which have similar solubilities and reactive

groups, in a mixture, are difficult, if not impossible, to separate. The usual method for the assay of mixed barbiturates consists of determining the total amount gravimetrically and assuming that each is present in the proper proportion.

Shill (1) and Bar (2) used the bromination procedure for determining barbiturates which contain an unsaturated radical. The technics employed by these authors differed considerably, but the reactions involved were essentially the same.

Ribley, *et al.*, (3) proposed a method for the simultaneous determination of sodium propylmethylcarbinylallyl barbiturate and sodium isoamylethyl barbiturate by use of the infrared spectrophotometer. These authors obtained exceptionally good results, but use of the method is limited to those laboratories which have the necessary special equipment. Also, the scope of the investigation did not extend beyond these two barbiturates. Umberger and Adams (4) also determined, for the most common barbiturates, the infrared absorption spectra which were then used to identify the drugs in toxicological examinations.

The purpose of this paper is to describe a rapid and convenient method for the determination and identification of some alkene barbiturates, either individually or in mixtures. When one constituent contains an allyl or cyclopentenyl radical, either of these components can be determined quantitatively by bromination and the other can be calculated by difference. Also, the solubility of the brom-derivative is such that it can be separated, purified by recrystallization, and identified by the melting point. The thiobarbiturates can also be determined by a similar procedure in which the sulfur is oxidized to sulfate with standard bromine solution. Preliminary investigation demonstrated that the addition of free bromine to the allyl and cyclopentenyl radicals was so nearly instantaneous as to permit direct titration of these barbiturates. Each of these radicals adds two atoms of bromine, and the thio group reacts with eight.

EXPERIMENTAL

Total Barbiturates.—A quantity of the sample representing 0.25–0.3 g of alkene barbiturate or 0.1 g of thiobarbiturate was accurately weighed and transferred to a 125 ml separator. The total barbiturates were determined by the A.O.A.C. method (5), except that the residues were dried to constant weight by heating for 30-minute intervals at 80–85°C., without successive additions and evaporations of anhydrous ether. In the determinations of mixed secobarbital and amobarbital, the A.O.A.C. method was further modified, as follows: Two successive volumes of anhydrous ether were added and evaporated. The residue was dissolved in 5 ml of boiling anhydrous ether, 10 ml of petroleum ether (boiling range 35–65°C.) was added, and the mixed solvents were evaporated to dryness. The residue was dried to constant weight, as described above.

Alkene Barbiturates.—The weighed residue from the determination of total barbiturates was dissolved in 10–15 ml of methanol, and 5 ml of HCl (1+1) was added. The solution was chilled in ice water to ca 10°C. and rapidly titrated, with

stirring, with 0.1 *N* KBr–$KBrO_3$ solution to a faint yellow color that persisted for 30 seconds. Approximately 1 ml of 10% KI solution was added, and the liberated iodine was titrated with 0.1 *N* $Na_2S_2O_3$.

Thio Barbiturates.—The residue was dissolved in 10 ml of 0.5 *N* NaOH and transferred quantitatively to an iodine flask; 50 ml of 0.1 *N* Br–$KBrO_3$ and 5 ml of $CHCl_3$ were added, and the solution was acidified with 10 ml of HCl (1+1). The flask was immediately tightly stoppered and allowed to stand for 1 hour with occasional shaking; it was then chilled in ice water to reduce the pressure, and 5 ml of 10% KI solution was added in the usual manner. The liberated iodine was titrated with 0.1 *N* $Na_2S_2O_3$, and starch T. S. was added as indicator near the end point.

Equivalence values of some alkenyl barbiturates and thiobarbiturates are as follows:

	g/ml
Allyl barbituric acid	0.01121
Allyl-cyclopentenyl barbituric acid	0.00586
Aprobarbital	0.01051
Diallyl barbituric acid	0.00521
Secobarbital	0.01191
Thiopental	0.003185

Other Barbiturates.—Barbiturates other than alkenyl or thiobarbiturates were calculated from the difference between total barbiturates and the alkenyl or thio component.

IDENTIFICATION OF THE ALKENYL BARBITURATE

The reaction mixture from the titration was transferred to a 125 ml separator. The beaker was rinsed with two 25 ml portions of ether, and the rinsings were added to the separator. The separator was shaken for about 1 minute, and the aqueous phase was drawn off into a second separator and extracted with an additional 30 ml portion of ether. The aqueous phase was discarded, and the two ether layers were washed successively with 10 ml of H_2O which contained enough $Na_2S_2O_3$ to discharge iodine color in the ether layer. Washing of the ether layers with H_2O was continued until the washings contained no more than a trace of halide. The ether was filtered through cotton into a beaker and was evaporated to dryness on the steam bath. Approximately 10 ml of $CHCl_3$ was added and the solution was slowly boiled on the steam bath until all the residue was dissolved. The solubilities of the brom-derivatives vary over a wide range, and some of the compounds required more $CHCl_3$ and protracted boiling. Other brom-compounds, such as isobutyl-allyl and cyclopentenyl-allyl, required use of a mixture of $CHCl_3$ and C_2H_5OH; they were dissolved by suspending the residue in boiling $CHCl_3$ and slowly adding C_2H_5OH until solution was complete. The beaker was removed from the steam bath and allowed to stand with occasional stirring until a copious precipitate was formed. The precipitate was filtered by suction through a Gooch crucible fitted with a disk of filter paper, and the beaker and crucible were washed with three 5 ml portions of $CHCl_3$. Suction was continued until all the $CHCl_3$ was removed from the precipitate. Contents of the crucible were transferred to the original beaker and recrystallized from 50% C_2H_5OH. The precipitate was filtered as described above and was washed with three 10 ml volumes of H_2O. The product was dried at 100°C. and the melting point was determined.

DISCUSSION

Quantitative.—The bromination method, while not specific, affords a convenient and rapid method for the determination of allyl and cyclopentenyl barbiturates. This method may be modified for direct determination of these barbiturates in tablets or capsules, or as a check determination on the weighed residue. Thiobarbiturates do not react as rapidly because the reaction involves the oxidation of the thio group to sulfuric acid. The sulfur is replaced by oxygen and the corresponding substituted barbituric acid is formed, i. e., thiopental yields sulfuric acid and pentobarbital. Thiopental is almost insoluble in water, and chloroform was necessary for solution so that the reaction would proceed to completion.

An attempt was made to apply this method to vinbarbital, cyclohexenylbarbital, and hexobarbital, but the bromination did not proceed with sufficient rapidity to give quantitative results. The method used for thiobarbiturates was applied to these components, but the results were higher than theoretical.

The extent of the present investigation indicates that substitution and addition reactions are involved when vinbarbital and cyclohexenylbarbital are brominated.

At present the method also seems inapplicable to bromallyl barbiturates. The bromination appears to proceed normally, but upon the

TABLE 1.—*Results on direct bromation of some individual alkenyl barbiturates*

	WEIGHED, *g*	RECOVERY	
		g	*per cent*
Secobarbital	0.2530	0.2526	99.8
	0.2358	0.2353	99.8
	2.2833	0.2834	100.1
Aprobarbital	0.1505	0.1495	99.3
	0.2830	0.2827	99.9
	0.2992	0.2989	99.9
Allyl-cyclopentenyl barbituric acid	0.2393	0.2386	99.8
	0.2342	0.2355	99.7
	0.2640	0.4151	99.6
Allyl barbituric acid	0.2163	0.2153	99.6
Diallyl barbituric acid	0.2418	0.2402	99.3
Thiopental	0.1073	0.1068	99.5
	0.1029	0.1018	98.9
	0.1062	0.1059	99.7

addition of potassium iodide, the reaction is reversed. The added bromine is liberated, and in turn liberates iodine almost as fast as it can be titrated. After approximately one-half of the added bromine is liberated, the rate of this reaction decreases.

The results obtained on some individual alkenyl barbiturates by the bromination method are given in Table 1. In Table 2 are presented results

TABLE 2.—*Results on determinations of mixed alkenyl and alkyl barbiturates*

SECOBARBITAL			AMOBARBITAL BY DIFFERENCE			TOTAL BARBITURATES
WEIGHED	RECOVERY		WEIGHED	RECOVERY		
g	*g*	*Per cent*	*g*	*g*	*Per cent*	*Per cent recovered*
0.2438	0.2428	99.1	0.2552	0.2558	100.2	99.9
0.2586	0.2564	98.8	0.2594	0.2611	100.7	99.9
0.2464	0.2439	99.0	0.2455	0.2478	100.9	100.0
0.2613	0.2612	100.0	0.2606	0.2602	99.8	99.9
0.2744	0.2731	99.5	0.2748	0.2767	100.7	100.1
0.2480	0.2487	100.3	0.2494	0.2495	100.0	100.2
0.2647	0.2666	100.7	0.2648	0.2624	99.1	99.9
0.2608	0.2612	100.2	0.2612	0.2610	99.9	99.9
0.2493	0.2493	100.0	0.2487	0.2477	99.6	99.6
0.2596	0.2606	100.4	0.2599	0.2573	99.0	99.7
0.2636	0.2642	100.2	0.2647	0.2634	99.5	99.9
0.2677	0.2683	100.2	0.2683	0.2672	99.6	99.9
0.2758	0.2764	100.2	0.2745	0.2734	99.6	99.9
0.2789	0.2796	100.2	0.2791	0.2781	99.6	99.9
0.2435	0.2427	99.7	0.2291	0.2302	100.5	100.1
Av.		100.0			99.9	99.9
Aprobarbital			*Phenobarbital*			
0.2048	0.2037	99.5	0.2070	0.2072	100.1	99.8
Allylbarbituric Acid			*Butabarbital*			
0.2086	0.2079	99.7	0.2139	0.2145	100.3	100.0
Diallylbarbituric Acid			*Phenobarbital*			
0.2357	0.2343	99.4	0.2181	0.2195	100.6	100.0
Allyl-cyclopentenyl Barbituric Acid			*Phenobarbital*			
0.2275	0.2264	99.5	0.2080	0.2093	100.6	99.8

of the determination of alkenyl barbiturates in the presence of alkyl barbiturates.

Qualitative.—Some of the brominated barbiturates are so insoluble in chloroform that a mixture of chloroform and ethanol is necessary to dissolve them in a reasonable amount of solvent. Some are difficult to dissolve and, when once in solution, are rather hard to precipitate. The use of this apparent insolubility of the brom-derivatives was investigated as a

means of separating the unsaturated from the saturated barbiturates for identification of each by melting points. However, when some of the pure unsaturated barbiturates were brominated, two different compounds were formed. One of the compounds is very slightly soluble in chloroform; the other component is very soluble in most organic solvents. This phenomenon precludes the use of solubilities for the convenient separation of the saturated barbiturate in pure form. The freely soluble fraction of the brom-derivative of secobarbital increases the solubility of the slightly soluble fraction; this causes the first crystallization to be more difficult than the second. The two derivatives were separated from some of the alkenyl barbiturates, and were recrystallized from chloroform until a constant melting point was obtained. The two brom-compounds derived from any particular allyl barbiturate have distinctively different melting points. The derivatives which were recrystallized from chloroform until a constant melting point was obtained were again recrystallized from approximately 50 per cent alcohol. Crystallization from the latter solvent resulted in a product with a melting point about 6° higher than that obtained when chloroform was used. When secobarbital was brominated, the chloroform-insoluble fraction was crystalline; the soluble fraction was amorphous and failed to crystallize from the common organic solvents, even at temperatures below 0°C.

An attempt was made to determine bromine in the various brom-derivatives investigated, but consistent results were not obtained. Additional investigation was not undertaken on the brom-derivatives of unsaturated barbiturates because such information would not particularly serve the purpose of this paper. If time permits, this investigation will be continued from the standpoint of composition, possible isomerism, and factors influencing the reaction.

In Table 3 are given data for the melting points of the brom-derivatives of some alkenyl barbiturates, some of which were also reported by Bar (2). The melting points listed in this paper are considerably higher than

TABLE 3.—*Melting points of brom-derivatives of some alkenyl barbiturates*

BARBITURIC ACID DERIVATIVE	$CHCl_3$ INSOL.		$CHCl_3$ SOL.		FROM LITERATURE
	FROM $CHCl_3$	FROM H_2O-C_2H_5OH	FROM $CHCl_3$	FROM H_2O-C_2H_5OH	
Methyl Butyl Allyl	196–200	200–204	——	——	——
Isobutyl Allyl	261–263	267–267.5	174–175	186	242
Isopropyl Allyl	218–220	224–226	148–150	165–166	195
Cyclopentenyl Allyl	248–249*d*	254–257*d*	—	—	204
Allyl Allyl	214–217	—	—	—	216

those found by Bar, with the exception of the brom-derivative of diallylbarbiturate. The lower melting points reported by Bar may be the result of a mixture of the two brom-derivatives.

The solubility in chloroform of some of the least soluble brom-barbiturates was determined. The determinations were made by dissolving the compounds in hot chloroform and allowing the solution to stand with an excess of solute in a glass-stoppered flask for twenty-four hours at room temperature (about 25° C.). The solutions were then filtered and measured volumes were evaporated to dryness on the steam bath. The residues were dried to constant weight at 100° C. Values for the solubilities are given in Table 4.

TABLE 4.—*Approximate solubility of some brom-alkenylbarbiturates in $CHCl_3$*

DERIVATIVE	SOLUBILITY AT R.T., G/100 ML
Methyl Butyl Allyl	0.31
Isobutyl Allyl	0.006 0.0064
Cyclopentenyl Allyl	0.009
Allyl Allyl	0.02

SUMMARY

A bromination method is described for the determination of some alkenyl and thiobarbiturates, both alone and mixed with other barbiturates. A method is also described for the separation and identification of the brom-barbiturates by their melting points. Tables of quantitative results and melting points of the brom-derivatives are given. The solubilities of some brom-derivatives in chloroform are also given.

REFERENCES

(1) SHILL, G., *Svensk. Farm. Tid.*, **50**, 385 (1946).
(2) BAR, D., *Bull. Soc. Pharm. Lille*, No. **2**, 16 (1948).
(3) RIBLEY, A. M., KENNEDY, E. E., HILTY, W. W., and PARK, T. V., *J. Am. Pharm. Assoc.*, **40**, 572 (1951).
(4) UMBERGER, C. J., and ADAMS, G., *Anal. Chem.*, **24**, 1310 (1952).
(5) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington, D. C., 1950, p. 590.

SEPARATION AND IDENTIFICATION OF CHLORINATED ORGANIC PESTICIDES BY PAPER CHROMATOGRAPHY. IV. THE COMPONENTS OF DILAN

By LLOYD C. MITCHELL (Division of Food, Food and Drug Administration, Department of Health, Education and Welfare, Washington 25, D. C.)

This paper describes the separation and identification by paper chromatography of the components of Dilan[1] which consists primarily of one part Prolan[1] and two parts Bulan.[1]

METHOD

APPARATUS

The chromatographic tank and accessories have been previously described (1, 2, 4).

REAGENTS

(a) *Immobile solvent.*—Dimethylformamide, 15 ml dild to 100 ml with ethyl ether, A.C.S. grade.

(b) *Mobile solvent.*—Mixed octanes,[2] commercial grade.

(c) *Chromogenic agents.*—(1) To 11 g KOH, add 5 ml H_2O; swirl until dissolved, cool, and dil. with methanol to 100 ml. (2) To 1 g $FeSO_4 \cdot 7H_2O$, add ca 50 ml H_2O; swirl, add 20 ml HCl, and dil. with H_2O to 100 ml.

(d) *Standards.*—Prolan, Bulan, and Dilan; 0.1 *M* solns in ethyl acetate.

(e) *Filter paper.*—Whatman No. 1, 8×8 in. sheets.

PROCEDURE

Spot 8×8 in. paper with 0.005 ml portions of the 0.1 *M* solns at equal intervals along base line; spray paper with immobile solvent and develop chromatogram as previously described (2, 3). When mobile solvent approaches top of sheet (ca 1¼ hrs.), remove paper from tank, mark solvent front, and hang from rod in hood until apparently dry (ca 3 min.). Wearing rubber gloves, spray paper with chromogenic agent c–1, allow to air dry (ca 10 min.), and then spray with agent c–2. Mark spots immediately.

DISCUSSION

The chromogenic agents previously used to detect chlorinated pesticides (3, 4) failed to react with the components of Dilan. Scott and Treon (5) have stated that ferric chloride will react with 2-nitropropane and 2-nitrobutane to produce colors which fade rapidly. With paper chromatograms, ferric chloride was an unsatisfactory reagent for Prolan and Bulan. Ferrous sulfate, applied as described under PROCEDURE, consistently produced a brown spot that persisted for several minutes even

[1] Trade mark names registered by Commercial Solvents Corporation, Terre Haute, Ind. Prolan is 2-nitro-1,1-bis(*p*-chlorophenyl)propane. Bulan is 2-nitro-1,1-bis(*p*-chlorophenyl)butane.

[2] Phillips Petroleum Co., Bartlesville, Okla.

with only one drop (0.005 ml) of 0.01 molar solutions (about 15 micrograms of sample). With more material (0.005 ml of 0.1 molar solutions), the spots sometimes were still visible after standing overnight. Ferrous iron alone, or sprayed prior to addition of the alkali, failed to produce a color. The R_F values for the 0.1 molar solutions of Prolan, Bulan, and Dilan are given in Table 1.

TABLE 1.—*R_F Values of Prolan, Bulan, and Dilan*[a]

COMPONENT	TEMPERATURE, ° C.	NO. OF OBSERVATIONS	R_F VALUES	
			AVERAGE	RANGE
Prolan	24	16	0.56	0.52–0.61
	25	16	0.55	0.53–0.58
Bulan	24	12	0.70	0.67–0.72
	25	12	0.68	0.66–0.70
Dilan	25	28	—	—
Prolan	—	—	0.52	0.50–0.57
Bulan	—	—	0.64	0.62–0.67

[a] Solvent system: immobile, dimethylformamide+ethyl ether, 15+85 v/v; mobile, mixed octanes.

SUMMARY

The two components of Dilan are separable by paper chromatography with the use of dimethylformamide as the immobile solvent and mixed octanes as the mobile solvent. They are detected on paper by spraying with methanolic potassium hydroxide, followed by a hydrochloric acid solution of ferrous sulfate.

REFERENCES

(1) MITCHELL, L. C., *This Journal*, **36**, 1187 (1953).
(2) *Ibid.*, **36**, 943 (1953).
(3) *Ibid.*, **35**, 920 (1952).
(4) MITCHELL, L. C., and PATTERSON, W. I., *ibid.*, **36**, 553 (1953).
(5) SCOTT, E. W., and TREON, J. F., *Ind. Eng. Chem., Anal. Ed.*, **12**, 189 (1940).

BOOK REVIEWS

Crime Investigation. By PAUL L. KIRK. Interscience Publishers, Inc., New York, 1953. 784 pp. Illus., index. Price $10.00.

The public has frequently been slow to accept scientific discoveries. This has been due in part to the failure of scientists to portray their discoveries in terms that are readily understood by the layman. Without the application of scientific methods it would be futile for law enforcement agencies to attempt to compete with our rapidly growing criminal element. The need for a book describing modern scientific crime investigation in relatively simple terms has long existed.

Dr. Paul L. Kirk's *Crime Investigation* will be valuable to a wide variety of readers. Those who are not scientifically trained will learn that practically every phase of the physical sciences is employed in examining physical evidence in criminal cases. The author makes it quite clear that the success or failure in solving a crime is largely dependent upon the ability and skill of the investigating officers and the laboratory scientists. A study of the methods actually employed will convince the reader that the quick solutions of crimes in many crime stories and radio plays are rather absurd. Police officers, attorneys, specialists and students in the field of crime investigation, and court reporters should find this book entertaining and informative.

Crime Investigation (766 pages plus a good index) is divided into two sections which may be studied independently. Section I, "Physical Evidence in General Criminal Investigations", discusses probability, samples and standards, the various items which may serve as evidence, instruments used in examining physical specimens, and the problem of identity. Perhaps of greatest significance is the emphasis placed on the training and the responsibilities of officers in recognizing, collecting, tabulating, and preserving materials which are to be delivered to the scientific laboratory for examinations.

In Section II, "Laboratory Operations and Techniques", the author covers many tests and technics employed in the examination of physical evidence. Even the skilled laboratory worker will benefit from some of Dr. Kirk's ideas and experiences. The author emphasizes the necessity of perfect cooperation between the investigating officers and the laboratory scientists.

This book was written to aid police investigators, students of criminalistics, and small police laboratories; it is not intended as a guide for specialists. A specialist in any phase of criminalistics would require far more scientific background and training than could be derived from the book alone. Forty-nine of the fifty-seven chapters are followed by bibliographies which afford easy location of additional studies for the serious student.

Two badly jumbled sentences, obviously due to type-setting errors, appear on pages 306 and 518.

The author has covered a wide variety of material which is applicable to scientific crime detection. A careful study of this book by police officers would doubtless improve their efficiency.

C. B. POLLARD

Advances in Agronomy. Volume V. A. G. NORMAN, Editor. Academic Press Inc., New York, 1953. x+422 pp. Price $8.50.

Volume V of this group follows the general pattern of the earlier books of the series and maintains the previous high standard. The first article, entitled "A Half Century of Wheat Improvement in the United States", occupies about one-third of the book and is, in effect, a magnificent monograph on the subject. Coverage

is historical as well as present-day factual. "The Organic Soil Fraction", one of the shorter chapters of the book, is a splendid condensation of important information. Historical aspects might, however, be strengthened by reference to work such as that of Shorey. Biochemists may wonder why no mention is made of the widely used term "carbon-nitrogen ratio."

A discussion of the agriculture and horticulture of Canada covers varied crops, climatic conditions, farm implements, and agricultural practices in that country. General trends indicate fuller utilization of forage crops in the future. A chapter on agricultural engineering in the United States covers many phases of a subject very important to the modern farmer. Greater use of the airplane is predicted for the next decade.

The chemical weathering of soils is partially summarized by a statement that inherent fertility of soils is related to their mineral content. As the weathering stage advances, soils gradually change, first toward increased productivity and finally to low productivity. A discussion of soil management indicates that it is much too early to make an accurate evaluation of the place that chemical soil conditioners will have as agents for maintenance of soil structure. These materials may some day serve to supplement the effects of soil organic matter.

A set of the five volumes of the series published thus far is a valuable addition to the library of a technical agriculturist, not only because of the information presented and discussed, but also for the references listed.

M. S. ANDERSON

Soils and Fertilizers. By FIRMAN E. BEAR. John Wiley and Sons, Inc., New York, 1953. xii+420 pp. Price $6.00.

It is a pleasure to read a distinctive and stimulating book such as the fourth edition of F. E. Bear's *Soils and Fertilizers*. The book approaches national coverage, but eastern agricultural conditions receive greatest emphasis. The western student will note an inadequate discussion of quality of irrigation water. This volume is definitely slanted toward fertilizers and plant nutrition, but general soil features are not neglected. Condensed but adequate treatments cover such topics as chemistry, physics, and biology of the soil in relation to the climatic conditions necessary to produce a crop.

The requirements of plants are discussed from several viewpoints, such as water, air, nitrogen, and various mineral elements. The nature and extent of our nation's resources, with respect to several nutrient elements, are presented as separate chapters of the book. Coverage of compounds present in soil and water provides useful information. The reader finds also abundant information regarding the crop requirements for different elements and the response that may be expected from additions of these elements to soil at varying rates. Fertilizer resources and their agronomic use are extensively discussed and are well illustrated. This work is brought up to date by inclusion of such subjects as "Direct Use of Anhydrous Ammonia", and "Combinations of Pesticides with Fertilizers".

In one chapter, entitled "Yield Potentialities of Crop Plants", the author presents a highly controversial topic in a concise manner. The college student would probably also welcome inclusion of some statements of the position taken by those who do not entirely agree with the conclusions reached by Mitscherlich and others.

Professor Bear has written a good book that should be welcomed by many college students as well as by practical agriculturists. For those who wish to delve further into the subject matter of particular chapters, good lists of selected references are provided.

M. S. ANDERSON

Condensed Pyridazine and Pyrazine Rings (Cinnolines, Phthalazines, and Quinoxalines). By J. C. E. SIMPSON. Interscience Publishers, Inc., New York, 1953. xvi+394 pages. Illus., index. Price $12.50 (By subscription: $11.25).

This volume, the fifth in a series of monographs on *The Chemistry of Heterocyclic Compounds,* is a valuable addition to the reference works now available to the chemist interested in this rather involved division of organic chemistry.

The references to, and the discussion of, two of the three major subjects of this book, cinnolines and phthalazines, are complete. The third major subject, quinoxalines, discussed earlier in such books as Meyer–Jacobson's *Lehrbuch der organischen Chemie,* is brought up to date by references to all the work reported in the period 1917–1948.

The scope of this work may well be visualized by an inspection of the following chapter headings: General Introduction to Cinnoline Derivatives. Preparation and Properties of Cinnoline; 4-Aryl-, 4-Acyl-, and 4-Carboxycinnolines; 4-Methylcinnolines; 4-Hydroxycinnolines; 4-Chloro-, 4-Alkoxy-, and 4-Phenoxycinnolines; 4-Aminocinnolines; Cinnoline Quaternary Salts; Reduced Cinnolines; Cinnolines Containing Additional Fused Rings; Phthalazines Unsubstituted in the Hetero Ring; 1-Alkyl-, 1-Aryl-, and 1,4-Diarylphthalazines; 1-Hydroxyphthalazines; Alkyl, Aryl, and Acyl Derivatives of 4-(1-)Hydroxyphthalazines; 1-Hydroxy-3-aryl-3,4-dihydrophthalazine-4-acetic Acids; 3-Aryl-1-ketophthalazines; Methylated Derivatives of 3-Aryl-1-ketophthalazines; 1,4-Dihydroxyphthalazines; Monoalkyl and Monoacyl Derivatives of 1,4-Dihydroxyphthalazines; Dialkyl and Diacyl Derivatives of 1,4-Dihydroxyphthalazines; 1-Halogeno- and 1,4-Diahalogenophthalazines; 1-Aminophthalazines; Reduced Phthalazines; Condensed Phthalazines and Azophthalazines; Preparation of Quinoxalines from Primary Aromatic *o*-Diamines and 1,2-Dicarbonyl Compounds; Quinoxalines Unsubstituted in the Hetero Ring; Quinoxaline *N*-Oxides; 2-Hydroxy- and 2,3-Dihydroxyquinoxalines; Quinoxaline-2-aldehydes; Quinoxaline-2-carboxylic and 2,3-dicarboxylic Acids; 2-Chloro- and 2,3-Dichloroquinoxalines; 2-Amino- and 2,3-Diaminoquinoxalines; 2-Alkoxy-, 2,3-Dialkoxy-, and 2,3-Diaryloxyquinoxalines; 2-Methyl- and 2,3-Dimethylquinoxalines; Quinoxaline Quaternary Salts; Formation of Quinoxalines from Compounds Containing a Furan Ring; 2-Polyhydroxyalkylquinoxalines; Reduced Quinoxalines; Condensed Quinoxalines; and Azaquinoxalines.

The book is completed by three appendices listing "Ultraviolet Absorption Spectra of Cinnoline and Quinoxaline Derivatives"; "Basic Strengths of Cinnoline, Phthalazine, and Quinoxaline Derivatives"; and "Antibacterial and Parasiticidal Activities of Cinnoline and Quinoxaline Derivatives".

The impressive mass of tabulated data which have been collected in this volume on the compounds mentioned above include structures, methods of preparation with occasional critical notes, physical characteristics, and references. The text deals both with general aspects of the various classes under discussion and with some details of descriptions of preparations and properties of various specific compounds.

Chemiluminescence of the phthalazine compounds is an example of a subject well treated by Simpson. The salient features of this activity are presented in a lucid manner with references given for each major point.

A large portion of the book is comprised of tables listing compounds and their properties, an arrangement which constitutes one of its most valuable features; yet this very strength points up a weakness in a reference text of this sort. Unfortunately, the book suffers, as do many others, in that the subject index is not as complete as the reader might wish.

This monograph is a very valuable adjunct for the chemist interested in these

nitrogen-containing heterocyclic compounds. It affords a reference work in a field which has been covered by no comparable predecessor.

SIDNEY M. HESS

Synthetic Organic Chemistry. By R. B. WAGNER and H. D. ZOOK. John Wiley and Sons, Inc., New York, 1953. xii+887 pp. Price $11.50.

With the intense activity in organic chemistry today, the organic chemist who spends most of his time in the laboratory can read only a portion of the literature. New synthetic methods, or valuable modifications of older ones, are constantly being discovered. In many instances these changes either make possible a reaction previously impossible or increase the yield to a practical range. The reduction of carbonyl groups with lithium aluminum hydride is a well known example.

The authors have covered the major sources of synthetic organic chemistry from 1919 to 1950 and have organized the information on mono- and difunctional compounds into a readily usable form in one volume. In such limited space, obviously, preparative details could not be included; however, there are abundant references, selected "for clarity of directions along with statements of yield and physical constants." Catalysts, abnormal reactions, or unusual conditions are mentioned.

Each main class of organic compounds is given a chapter in this book. The tables at the end of each chapter are further subdivided; e.g., hydroxy, dihydroxy, hydroxy olefins, hydroxy acetylenes, hydroxy halides, hydroxy ethers. At the beginning of each chapter the methods to be described are tabulated and each one is given a method number; in the tables, only this number is listed in the method column. The compounds in each table are listed in order of increasing carbon content and for every compound there are given the method number, yield, chapter reference, boiling point or melting point, refractive index, and sometimes a derivative. A total of 576 reactions are discussed. The index does not include the compounds listed in the tables, but emphasizes individual organic reactions.

This book contains so much information about the synthesis of organic compounds that every active worker in the field can save much time by having it on his desk. A companion volume covering compounds beyond the difunctional ones would be most welcome.

W. I. PATTERSON

The Statistics of Bioassay, with Special Reference to the Vitamins. By CHESTER I. BLISS. Academic Press, Inc., New York, 1952. 184 pp. Price $3.50.

This book is a seprate printing, complete even to the original page numbers (445–628) of the chapter "Statistical Methods in Vitamin Research", contained in *Vitamin Methods, Volume II*, edited by Dr. Paul Gyorgy and published in 1951. Minor corrections have been made and some additional material has been included.

The reprinting was almost preordained, since the statistical methods presented pertain to bioassay in general and are not restricted to the special field of vitamins. No attempt has been made to describe mathematical derivations; instead, the operating procedures, the necessary equations, and the interpretation of the results are given, together with examples completely worked out.

This volume is an invaluable addition, as a ready reference, to the library of the biologist and others concerned with biological assay; it deserves wide distribution.

WILLIAM WEISS