

UC-NRLF



TABLE XLVI.—Maize—individual analyses.

[Pounds per hundred pounds of dry matter.]

Lab- ora- tory No.	Year and crop.	Water.	Dry matter.	Ash.	Protein (N X 6.25).			Fat.			Crude fiber.			Carbohydrate.			Total production value.		Nutri- tive ratio.
					Total.	Digest- ible.	Produc- tion value— gain in flesh.	Total.	Digest- ible.	Produc- tion value— gain in flesh.	Total.	Digest- ible.	Produc- tion value— gain in flesh.	Total.	Digest- ible.	Produc- tion value— gain in flesh.	Gain in flesh.	Calorific.	
983	1906	12.98	87.02	1.56	9.69	6.98	1.64	4.13	3.68	1.94	2.19	1.27	82.43	78.31	19.42	23.31	100,769	12.6	
984	1906	12.84	87.16	1.47	9.25	6.66	1.57	4.89	4.35	2.29	2.25	1.31	82.14	78.03	19.35	23.53	101,720	13.4	
985	1906	13.09	86.91	1.47	8.50	6.12	1.44	4.59	4.09	2.15	2.51	1.46	82.03	78.78	19.54	23.49	101,547	14.6	
986	1906	13.17	86.83	1.52	9.69	6.98	1.64	4.72	4.20	2.20	2.31	1.34	81.76	77.67	19.26	23.43	101,287	12.6	
987	1906	13.15	86.85	1.54	8.50	6.12	1.44	4.21	3.75	1.97	2.10	1.22	83.65	79.46	19.71	23.42	101,245	14.5	
988	1906	13.08	86.92	1.54	6.98*	6.98	1.64	4.23	3.76	1.98	1.85	1.07	82.69	78.55	19.48	23.36	100,985	12.6	
989	1906	12.88	87.11	1.63	11.19	8.06	1.89	4.74	4.22	2.22	2.07	1.20	80.48	76.45	18.96	23.37	101,028	10.8	
990	1906	12.89	87.11	1.63	12.19	8.78	2.06	4.65	4.14	2.18	2.33	1.35	79.20	73.24	18.66	23.23	100,423	9.8	
991	1906	13.15	86.85	1.45	8.88	6.39	1.50	4.28	3.81	2.00	2.36	1.37	83.03	78.87	19.56	23.40	101,158	13.9	
992	1906	13.38	86.62	1.55	12.25	8.82	2.07	3.54	3.15	1.18	2.04	1.18	80.62	76.59	18.99	23.01	99,472	9.6	
993	1906	13.30	86.70	1.38	10.19	7.34	1.72	4.31	3.84	2.02	2.20	1.28	81.92	77.82	19.30	23.36	100,985	11.9	
994	1906	12.79	87.21	1.58	8.94	6.44	1.51	4.52	4.02	2.11	2.27	1.32	82.69	78.55	19.48	23.43	101,287	13.8	

1909

Issued January 25, 1909.

U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF CHEMISTRY—BULLETIN No. 121.  
H. W. WILEY, Chief of Bureau.

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# FOOD LEGISLATION DURING THE YEAR ENDED JUNE 30, 1908.

BY

W. D. BIGELOW,  
*Chief, Division of Foods,*

WITH THE COLLABORATION OF

N. A. PARKINSON.



WASHINGTON:  
GOVERNMENT PRINTING OFFICE.  
1909.

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Nashville. [Not appointed.]

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Issued January 25, 1909.

U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF CHEMISTRY—BULLETIN No. 121.  
H. W. WILEY, Chief of Bureau.

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FOOD LEGISLATION DURING THE YEAR  
ENDED JUNE 30, 1908.

BY

W. D. BIGELOW,  
*Chief, Division of Foods,*

WITH THE COLLABORATION OF

N. A. PARKINSON.



WASHINGTON:  
GOVERNMENT PRINTING OFFICE.  
1909.

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## LETTER OF TRANSMITTAL.

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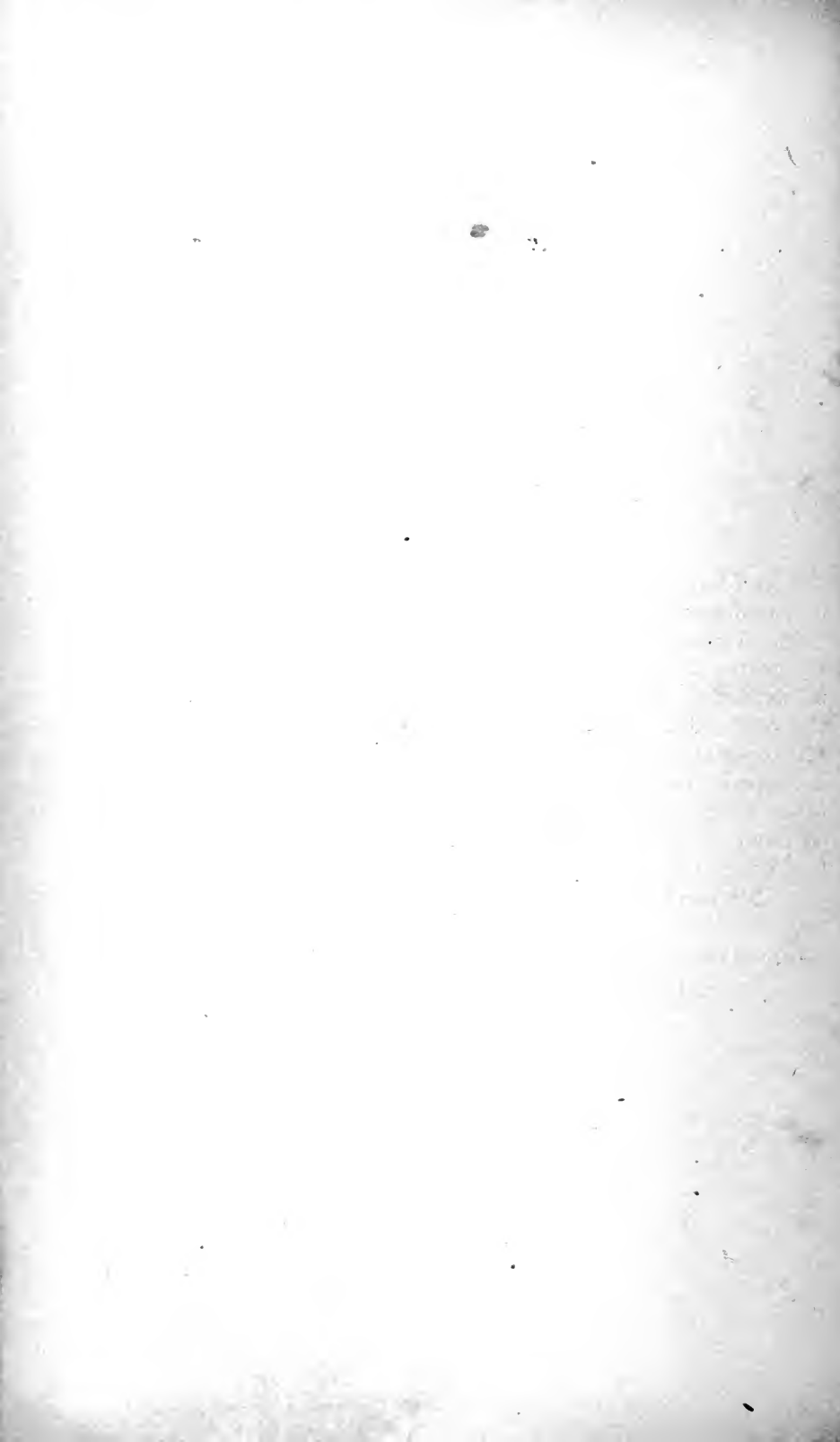
U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF CHEMISTRY,  
*Washington, D. C., November 30, 1908.*

SIR: I have the honor to transmit for your inspection and approval a compilation of the food legislation during the year ended June 30, 1908. I recommend its publication as Bulletin 121 of the Bureau of Chemistry, supplementing Bulletin 112, Parts I and II, Food Legislation during the Year ended June 30, 1907; Bulletin 104; covering the year ended June 30, 1906, and Bulletin 69, Revised, Parts I to IX, covering all legislation prior to July 1, 1905. This compilation is of special value at this time, when so many changes are being made in the State laws tending toward uniformity with the National food law, and in view of the close cooperation between State and Federal food officials.

Respectfully,

H. W. WILEY,  
*Chief of Bureau.*

HON. JAMES WILSON,  
*Secretary of Agriculture.*





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# FOOD LEGISLATION DURING THE YEAR ENDED JUNE 30, 1908.

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## FEDERAL LAWS.

During the fiscal year ended June 30, 1908, no Federal food laws were passed with the exception of the amendment to the tea law, which is printed herewith. The following orders, regulations, and decisions issued by the Bureau of Animal Industry and the Board of Food and Drug Inspection of the United States Department of Agriculture and by the Bureau of Internal Revenue of the United States Treasury Department are enumerated as a matter of record, but are not reprinted:

Bureau of Animal Industry: B. A. I. Order No. 147, Regulations Prescribed in Regard to Renovated Butter in Accordance with the Act of Congress Approved May 9, 1902 (July 11, 1907). B. A. I. Order No. 150, Regulations Governing the Meat Inspection of the United States Department of Agriculture (effective April 1, 1908). Amendment to B. A. I. Order No. 150, Amendment to Section 19 of Regulation 25, Exempting Shipments of Certain Inedible Grease, Tallow, or Other Fat from the Provision Requiring the Denaturing of Inedible Products (April 24, 1908). B. A. I. Meat Inspection Rulings—2 A, Colors (September 2, 1907). B. A. I. Meat Inspection Rulings—3 A, Notice Regarding the Enforcement of that Portion of Paragraph 5 of Section 19 of Regulation 25 of B. A. I. Order 150, Relating to the Denaturing of Inedible Grease, Tallow, and Other Fat (April 6, 1908).

Board of Food and Drug Inspection: Food Inspection Decision 74, Certificates for Imported Meats and Meat-Food Products of Cattle, Sheep, Swine, and Goats. 75, The Labeling of Mixtures of Cane and Maple Sirups. 76, Dyes, Chemicals, and Preservatives in Foods. 77, Certificate and Control of Dyes Permissible for Use in Coloring Foods and Foodstuffs. 78, The Use of Labels after October 1, 1907. 79, Collection of Samples. 80, Glazed Coffee. 81, Labeling of Caramels. 82, Labeling of Coffee Produced in the Dutch East Indies. 83, The Issue of a Guaranty Based upon a Former Guaranty. 84, Amendments to Regulations 17 and 19. 85, Labeling of Bitters. 86, Original Packages: Interpretation of Regulation 2 of Rules and Regulations for the Enforcement of the Food and

Drugs Act. 87, Labeling of Corn Sirup. 88, Private Importations. 89, Amendment to Food Inspection Decision 76, Relating to the Use in Foods of Benzoate of Soda and Sulphur Dioxid. 90, The Labeling of Foods and Medicinal Mixtures for Stock and Poultry. 91, The Labeling of Mocha Coffee. 92, The Use of Copper Salts in the Greening of Foods. 93, Amendment to Regulation 34. 94, The Labeling of Medicinal and Table Waters. 95, The Use of Neutral Spirits Distilled from Beet Sugar Molasses in the Preparation of Whisky Compounds and Imitation Whiskies. 96, Serial Number Guaranty.

Bureau of Internal Revenue: Regulation No. 9, Revised July, 1907, Revised Regulations Concerning Oleomargarine, also Adulterated Butter and Process or Renovated Butter.

#### TEA.

SEC. 1. *Importation of inferior tea prohibited; proviso.* From and after May first, eighteen hundred and ninety-seven, it shall be unlawful for any person or persons or corporation to import or bring into the United States any merchandise as tea which is inferior in purity, quality, and fitness, for consumption to the standards provided in section three<sup>a</sup> of this act, and the importation of all such merchandise is hereby prohibited. *Provided,* That nothing herein shall affect or prevent the importation into the United States, under such regulations as the Secretary of the Treasury may prescribe, of any merchandise as tea which may be inferior in purity, quality, and fitness for consumption to the standards established by the Secretary of the Treasury, or of any tea waste, tea siftings, or tea sweepings, for the sole purpose of manufacturing theine, caffeine, or other chemical products whereby the identity and character of the original material is entirely destroyed or changed; and that importers and manufacturers who import or bring into the United States such tea, tea waste, tea siftings, or tea sweepings shall give suitable bond, to be approved as to amount and securities by the Secretary of the Treasury, conditioned that said imported material shall be only used for the purposes herein provided, under such regulations as may be prescribed by the Secretary of the Treasury.—*As amended May 16, 1908. Statutes of the United States of America, 1907-1908, Part 1, ch. 170, p. 163.*

Approved March 2, 1897. United States Statutes at Large, 1895-1897, vol. 29, ch. 358, pp. 604-607.

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<sup>a</sup> See Bul. 69, Revised, Part I, p. 7.

## CANADA.

The statutes of Canada were not available and only those laws are included of which copies could be procured from the enforcing officer.

### MEAT AND CANNED GOODS.

**SEC. 1. Short title.** This Act may be cited as *The Meat and Canned Foods Act*.

**SEC. 2. Definitions.** In this Act, unless the context otherwise requires,

(a) "carcases" means the carcasses of cattle, swine, sheep, goats or poultry;  
(b) "establishment" means any abattoir, packing house, or other premises in which such animals are slaughtered, or in which any parts thereof or products thereof, or fish, or fruit, or vegetables, are prepared for food for export or are stored for export;

(c) "export" means export out of Canada, or out of the province in which the establishment is situated to another province;

(d) "food" includes every article used for food or drink by man, and every ingredient intended for mixing with the food or drink of man for any purpose;

(e) "inspector" means an inspector appointed under this Act;

(f) "Minister" means the Minister of Agriculture;

(g) "regulations" means regulations made under the provisions of this Act.

**SEC. 3. Inspection of animals.** All animals intended for slaughter in any establishment shall be inspected as provided by the regulations.

2. No animal shall be allowed to enter the parts of an establishment where slaughtering is carried on, unless it has undergone such inspection and been found to be healthy and fit for food.

3. Every animal affected, or suspected of being affected, with contagious or other disease, shall be slaughtered under the supervision of the inspector and be disposed of as provided by the regulations.

**SEC. 4. Inspection of carcasses.** All carcasses and portions thereof of all animals, wherever slaughtered, intended for export, shall be inspected as provided by the regulations.

**SEC. 5. Slaughtering by farmers and retail butchers.** Unless the Minister otherwise directs, upon the report of an inspector, animals owned by farmers and slaughtered by them on their own premises, and animals slaughtered by retail butchers on their own premises, shall not be subject to inspection under the provisions of this Act.

**SEC. 6. Healthy carcasses; marks on.** Every carcass, or portion thereof, found to be healthy and fit for food, shall be marked by the inspector in such manner as is provided by the regulations; and the carcass, or portion thereof, may then be dealt with as the owner thereof sees fit, subject to the further supervision of the inspector.

**SEC. 7. Inspection and marking of meat products.** Every carcass or portion or product thereof prepared for food in any establishment and packed in cans or similar receptacles, or in any package whatever, shall be subject to inspection during the whole course of preparation and packing; and after all the requirements of this Act regarding inspection have been complied with, and not until then, all such packages shall be marked by the inspector in such manner as is provided by the regulations.

**SEC. 8. Re-inspection.** The inspector may at any time re-inspect a carcass, or any portion or product thereof, in order to ascertain whether, subsequently to the first inspection thereof, it has undergone decomposition, or has otherwise deteriorated, or has been tampered with or adulterated by the use of preservatives or otherwise.

2. Every carcass, or portion or product thereof, sent out of an establishment, and returned thereto for any purpose, shall not be again sent out therefrom without re-inspection.

SEC. 9. *Unhealthy meat, disposal of.* Every carcass, or portion or product thereof, found, upon inspection or re-inspection, to be unhealthy or unfit for food, or which contains such ingredients or preservatives as may render it unfit for food, shall be marked by the inspector in such manner as is provided by the regulations, and shall thereupon be deemed to be condemned as unfit for food and shall be disposed of as provided by the regulations.

SEC. 10. *Sale, etc., of unhealthy meat.* Any person slaughtering, or permitting the slaughtering of, animals and selling, or offering for sale or transportation, for export a carcass, or any portion or product thereof, which is unhealthy or unfit for food is guilty of an indictable offence and liable to one year's imprisonment.

2. Every one who is convicted of this offence after a previous conviction for the same crime shall be liable to two years' imprisonment.

SEC. 11. *Exemption from inspection.* The Governor in Council may, upon application of the owner thereof, exempt any establishment from the operation of the provisions of sections 3 and 4, and of sections 6 to 10, both inclusive, of this Act.—*As amended June 16, 1908. 7-8 Edward VII, ch. 47.*

SEC. 12. *Inspection and marking of packages.* All articles prepared for food in any establishment and packed in cans or similar receptacles, or in any package whatever, shall be subject to inspection during the whole course of preparation and packing; and all such packages shall be marked with—

(a) the initials of the Christian names, the full surname, and the address, or, in the case of a firm or corporation, the firm or corporate name and address, of the packer; or of the first dealer obtaining them direct from the packer who sells or offers the said articles for sale; and such dealer shall, upon the request of an inspector appointed under this Act, disclose the name of the packer of such article.—*As amended June 16, 1908. 7-8 Edward VII, ch. 47.*

(b) a true and correct description of the contents of the package:

Provided, however, that if it be established to the satisfaction of the Governor in Council that such marking would hinder the sale of any of said articles in the British or foreign markets, he may exempt such articles from the provisions of this section.

SEC. 13. *Fish, fruit and vegetables.* All fish, fruit, or vegetables used in any establishment where these articles are prepared for export, shall be sound, wholesome, and fit for food; and any such articles or products thereof found in the said establishment unsound or unwholesome shall be confiscated and destroyed as provided by the regulations.

SEC. 14. *Sanitary conditions.* An inspection and close supervision of the sanitary conditions of any establishments shall be maintained as provided by the regulations.

2. The inspector shall refuse to inspect or mark articles in any establishment where the sanitary conditions are not in accordance with the regulations.

SEC. 15. *Withdrawal of inspector and closing of establishment for violation of Act, etc.* In the event of the provisions of this Act, or any regulations, or the lawful instruction of an inspector not being complied with in any establishment, the Minister may withdraw the inspector therefrom, and may refuse to it the inspection, marking, and certification of the articles prepared therein, and may cause the establishment to be closed.

SEC. 15A. *Sale in violation of Act.* No person shall offer or expose or have in his possession for sale any article subject to inspection under this Act unless all the requirements thereof respecting the said article have been complied with.—*Added June 16, 1908. 8-7 Edward VII, ch. 47.*

**SEC. 16. *Export of uninspected articles.*** No person shall offer or accept for export, or shall export, any articles subject to inspection under this Act, unless its requirements regarding inspection and marking have been complied with in respect to such articles.

2. No clearance shall be granted to any vessel carrying any carcasses, or any portions or products thereof, unless they are duly marked in accordance with the provisions of this Act.

3. The provisions of this section shall not apply to meats intended for consumption on board the vessels by which they are shipped from a Canadian port.

4. At the request of the owner of any establishment, the inspector in charge thereof shall issue certificates of inspection for any carcasses or portions or products thereof intended for export. Such certificates shall be in such form as is provided by the regulations.

5. Notwithstanding anything in this section, the Governor in Council may, whenever it is deemed necessary or advisable to do so, authorize the export of any such articles without inspection.

**SEC. 17. *False marking as to name, weight, and date.*** No article subject to inspection under this Act shall be offered or sold for export, or exported, under any name intended or calculated to deceive as to its true nature.

2. No package containing any article subject to inspection under this Act shall be marked with any label, brand or mark which falsely represents the quantity or weight or contents of such package.

3. No package containing any article subject to inspection under this Act shall be marked with any label, brand or mark which falsely represents the date when the articles or goods contained therein were packed.—*As amended June 16, 1908. 7-8 Edward VII, ch. 47.*

**SEC. 18. *Tampering with marks.*** Every person who, not being an inspector, wilfully alters, effaces, or obliterates, or causes to be altered, effaced or obliterated, wholly or partially, any mark on any article which has undergone inspection shall incur a penalty of one hundred dollars.

**SEC. 19. *Appointment of officers.*** The Minister may appoint inspectors and other officers for the carrying out of the provisions of this Act, but such appointments shall be confirmed by the Governor in Council within thirty days of the date thereof.

2. No person shall be appointed as a veterinary inspector until he has passed such examination as is deemed necessary by the Governor in Council.

**SEC. 20. *Regulations.*** The Governor in Council may make such orders and regulations, not inconsistent with the provisions of this Act, as to him seem necessary for the carrying out of the provisions of this Act.

2. Such orders and regulations shall have the same force and effect as if embodied in this Act.

3. Every such order or regulation shall be published twice in *The Canada Gazette*.

4. Any such order or regulation may be proved by the production of a copy thereof certified by the Minister; and such order or regulation shall, until the contrary is proved, be deemed to have been duly made and issued on the date thereof.

**SEC. 21. *Inspector's certificate as evidence.*** The certificate of the inspector or other officer appointed under the provisions of this Act shall, for the purpose of this Act, be prima facie evidence in all courts of justice and elsewhere of the matter certified.

**SEC. 22. *Inspector's power of entry.*** Any inspector or other officer appointed under the provisions of this Act may, at any time, for the purpose of carrying into effect any of the provisions of this Act, enter any place or premises, or

any steamship, vessel or boat, or any carriage, car, truck, horse-box or other vehicle used for the carriage of articles subject to the provisions of this Act, but shall, if required, state in writing the grounds on which he has so entered.

SEC. 23. *Obstructing inspector.* Every person who refuses to admit, or who obstructs or impedes, an inspector or other officer acting in execution of this Act, or of any order or regulation made by the Governor in Council or the Minister thereunder, and every person who aids and assists him therein, shall, for every such offence, incur a penalty not exceeding five hundred dollars; and the inspector or other officer may apprehend the offender and take him forthwith before a justice of the peace to be dealt with according to law; but no person so apprehended shall be detained in custody, without the order of the justice, longer than twenty-four hours.

SEC. 24. *Unlawful removal.* Every person who moves, or causes or allows to be moved, any animal, or any article in violation of the provisions of this Act, shall, for every such offence, incur a penalty not exceeding five hundred dollars.

SEC. 25. *Bribery of inspector.* The provisions of *The Criminal Code* respecting the bribery and corruption of officials or employees of the Government extend to all inspectors and other persons appointed to carry out the provisions of this Act.

SEC. 26. *Violations of Act.* Every person who violates any provision of this Act, or of any regulation made by the Governor in Council or by the Minister under the authority of this Act, in respect to which no penalty is hereinbefore provided, shall for every such offence, incur a penalty not exceeding five hundred dollars.

SEC. 27. *Apprehension of offenders.* Any inspector or constable may, without warrant, apprehend any person found committing an offense against the provisions of this Act, and shall take any person so apprehended forthwith before a justice of the peace to be examined and dealt with according to law; but a person so apprehended shall not be detained in custody, without the order of a justice, longer than twenty-four hours; and any inspector or constable may require that any animal or any article moved in violation of the provisions of this Act be forthwith taken back within the limits of the place whence it was moved, and may enforce and execute such requisition at the expense of the owner of such animal or article.

SEC. 28. *Place of committing of offence.* Every offence against this Act, or against any order or regulation of the Governor in Council or of the Minister, shall for the purpose of proceedings under this Act, or of any such order or regulation, be deemed to have been committed, and every cause of complaint under this Act, or any such order or regulation, shall be deemed to have arisen, either in the place in which it actually was committed or arose, or in any place in which the person charged or complained against happens to be.

SEC. 29. *Recovery of penalties.* Every penalty imposed by this Act shall be recoverable, with costs, before any two justices of the peace, or any magistrate having the powers of two justices of the peace, under Part XV. of *The Criminal Code*.—As amended June 16, 1908. 7-8 Edward VII, ch. 47.

SEC. 30. *Administration of Act.* The administration of any part of this Act may be assigned by the Governor in Council to any Minister other than the Minister of Agriculture, and in such case the Minister to whom such assignment is made shall have the same powers with respect to the part of this Act to him assigned as the Minister of Agriculture now has.

SEC. 31. *Suspension of operation.* The Governor in Council may suspend the operation of any of the sections of this Act until the first day of January next.

Assented to April 27, 1907. 6-7 Edward VII, ch. 27.



## **CONNECTICUT.**

See Appendix, Bulletin 112, Part I, page 152, for general food laws, passed July 31, 1907, and included in that publication for convenience, though the compilation covered only laws passed in the fiscal year ended June 30, 1907.

## KENTUCKY.

### GENERAL FOOD LAWS.

SEC. 1. *Adulterated or misbranded food unlawful; penalty; proviso.* That it shall be unlawful for any person, persons, firm or corporation within this State to manufacture for sale, produce for sale, expose for sale, have in his or their possession for sale or to sell any article of food or drug which is adulterated or misbranded within the meaning of this act; and any person or persons, firm or corporation who shall manufacture for sale, expose for sale, have in his or their possession for sale or sell any article of food or drug which is adulterated or misbranded within the meaning of this act shall be fined not less than ten dollars nor more than one hundred dollars, or be imprisoned not to exceed fifty days or both such fine and imprisonment. Provided, That no article of food or drug shall be deemed misbranded or adulterated within the provisions of this act when intended for shipment to any other State or country, when such article is not adulterated or misbranded in conflict with the laws of the United States; but if said article shall be in fact sold or offered for sale for domestic use or consumption within this State, then this proviso shall not exempt said article from the operations of any of the other provisions of this act.

SEC. 2. *Food defined.* That the term food, as used in this act, shall include every article used for or entering into the composition of food or drink for man or domestic animals, including all liquors.

SEC. 3. *Misbranding defined.* For the purpose of this act, an article of food shall be deemed misbranded:

First. If the package or label shall bear any statement purporting to name any ingredient or substance as not being contained in such article, which statement shall not be true in any part; or any statement purporting to name the substances of which such article is made, which statement shall not give fully the name or names of all substances contained in any measurable quantity.

Second. If it is labeled or branded in imitation of or sold under the name of another article, or is an imitation either in package or label of another substance of a previously established name; or if it be labeled or branded so as to deceive or mislead the purchaser or consumer with respect to where the article was made or as to its true nature and substance, or as to any identifying term whatsoever whereby the purchaser or consumer might suppose the article to possess any property or degree of purity or quality which the article does not possess.

Third. If in the case of certified milk, it be sold as or labeled "certified milk," and it has not been so certified under rules and regulations by any county medical society, or if when so certified it is not up to that degree of purity and quality necessary for infant feeding.

Fourth. If it be misrepresented as to weight or measure or, if where the length of time the product has been ripened, aged or stored, or if where the length of time it has been kept in tin or other receptacle, tends to render the article unwholesome, the facts of such excessive storage, ripening, aging or packing are not plainly made known to the purchaser and to the consumer.

**Fifth.** If the package containing it or its label shall bear any statement, design, or device regarding the ingredients or the substances contained therein, which statement, design or device shall be false or misleading in any particular. Provided, That articles of liquor which do not contain any added poisonous or deleterious ingredients shall not be deemed to be adulterated or misbranded within the provisions of this act, in the case of articles labeled, branded or tagged so as to plainly indicate that they are compounds, imitations, or blends, and the word "compound," "imitation," or "blend," as the case may be, is plainly stated on the package in which it is offered for sale. Provided, That the term blend as used herein shall be construed to mean a mixture of like substances, not excluding harmless coloring and flavoring ingredients used for the purpose of coloring and flavoring only.

**SEC. 4. Adulteration defined.** For the purpose of this act, an article of food shall be deemed to be adulterated:

**First.** If any substance or substances be mixed or packed with it so as to reduce, lower or injuriously affect its quality or strength.

**Second.** If any substance be substituted in whole or in part for the article.

**Third.** If any valuable constituent of the article has been wholly or in part abstracted; or if the product is below that standard of quality represented to the purchaser or consumer.

**Fourth.** If it is mixed, colored, coated, polished, powdered, or stained whereby damage is concealed, or if it is made to appear better or of greater value than it is, or if it is colored or flavored in imitation of the genuine color or flavor of another substance of a previously established name.

**Fifth.** If it contains added poisonous ingredient which may render such article injurious to health, or if it contains any antiseptic or preservative which may render such article injurious to health, or any other antiseptic or preservative not evident or not plainly stated on the main label of the package.

**Sixth.** If it consists of or is manufactured from in whole or in part of a diseased, contaminated, filthy or decomposed substance, either animal or vegetable, unfit for food, or an animal or vegetable substance produced, stored, transported or kept in a condition that would render the article diseased, contaminated or unwholesome, or if it is any part the product of a diseased animal, or the product of an animal that has died otherwise than by slaughter, or that been<sup>a</sup> fed upon the offal from a slaughterhouse, or if it is the milk from an animal fed upon a substance unfit for food for dairy animals or from an animal kept and milked in a filthy or a contaminated stable or in surroundings that would render the milk contaminated. Provided, That any article of food which may be adulterated and not misbranded within the meaning of this act, and which does not contain any added poisonous or deleterious ingredient and which is not otherwise adulterated within the meaning of paragraphs four, five and six of section four of this act, or which does not contain any filler or ingredient which debases without adding food value, can be manufactured or sold, if the same be labeled, branded or tagged so as to show the exact character thereof. And all such labels and all labeling of packages provided for in any provisions of this act shall be on the main label of each package and in such position and character of type and terms as will be plainly seen, read and understood by the purchaser or consumer. Provided further, That nothing in this act shall be construed as requiring or compelling the proprietors, manufacturers or sellers of proprietary foods which contain no unwholesome substances or ingredients to disclose their trade formulas except in so far as the provisions of this (act) require to secure freedom from adulteration, imita-

<sup>a</sup> So in Statutes.

tion or misbranding. But in the case of baking powders, every can or other package shall be labeled so as to show clearly<sup>a</sup> the name of the acid salt which shall be plainly stated in the face of the label to show whether such salt is cream of tartar, phosphate or alum. Provided further, That nothing in this act shall be construed to prohibit the manufacture or sale of oleomargarine, butterine, or kindred compounds in a separate and distinct form, and in such manner as will advise the consumer of the real character, free from coloration or ingredient that causes it to look like butter.

[Sections 5 to 7 relate to drugs.]

SEC. 8. *Director of experiment station to make analysis, fix methods and standards; board for establishing regulations.* It shall be the duty of the Director of the Kentucky Agricultural Experiment Station, or under his direction, the head of the division of food inspection of the said station, to make or cause to be made examinations of samples of food and drugs manufactured or on sale in Kentucky at such time and place and to such extent as he may determine. He shall also make, or cause to be made, analysis of any sample of food or drug which the State Board of Health or the State Board of Pharmacy may suspect of being adulterated or misbranded, and of any sample of food or drug furnished by any Commonwealth's, county or city attorney of this State. And the said director may appoint such agent or agents as he may deem necessary, who shall have free access at all reasonable hours for the purpose of examining into places wherein any food or drug product is being produced, manufactured, prepared, kept or offered for sale, for the purpose of determining as to whether or not any of the provisions of this act are being violated, and such agent or agents upon tendering the market price of any article may take from any person, firm or other corporation, a sample of any article desired for examination.

The director of said Experiment Station is hereby empowered to adopt and fix the methods by which the samples taken under the provisions of this act shall be analyzed or examined, and to adopt and fix standards of purity, quality or strength, when such standards are necessary or are not specified or fixed herein or by statute. Provided, That such standards shall be published for the information and guidance of the trade. Provided further, That for the purpose of uniformity, when such standards so fixed differ from the legally adopted standards of the United States Department of Agriculture, the director of said station shall arrange for a conference between the proper food control representatives of the United States Department of Agriculture and the director of said station and the representatives of the trade to be affected, for the purpose of arriving, if possible, at a uniform state and national standard. Provided further, That in the case of final dispute the validity of such standards adopted by the director of said station shall be determined by the Courts under the rules of evidence. And Provided further, That when the standard or nomenclature for any food or food product has been determined by the Supreme Court of the United States such standard or nomenclature shall govern in the enforcement of the provisions of this act. Provided further, That all rulings pertaining to sanitation under this act shall be collaborated in connection with the State Board of Health. And provided further, That at the regular annual meetings of the Kentucky Pharmaceutical association and the Kentucky State Medical association each of said associations shall elect one representative, which representatives, together with the director of said station shall make and establish all rules and regulations for the governing and carrying out of the provisions of this act relating to drugs.

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<sup>a</sup> So in Statutes.

**SEC. 9. Prosecution.** Whenever any article shall have been examined and found to be adulterated or misbranded in violation of this act, the Director shall certify the facts to the Commonwealth's attorney of the district, or to the county attorney of the county, or the city attorney of any city or town, in which the said adulterated or misbranded food or drug product was found, together with a statement of the results of the examination of said article of food or drug, duly authenticated by the analyst under oath and taken before some officer of this Commonwealth authorized to administer an oath having a seal. And it shall be the duty of every Commonwealth's attorney, county attorney and city attorney to whom the Director of said station shall report any violation of this act or to whom the State Board of Health, or the State Board of Pharmacy, or to whom the chief health officer of any county, city or town shall report any such violations, to cause proceedings to be commenced against the party so violating the act, and the same prosecuted in manner as required by law. Provided, however, That in case of the first charge or finding the manufacturer or dealer shall be notified of the findings and be given a hearing within fifteen days before a report is made to the Commonwealth's, county or city attorney as herein provided. Provided further, That where more than one sample of the same brand of product has been taken and examined, the first finding or charge shall be construed to apply to all samples so taken, and notice and hearing shall apply to all such samples.

**SEC. 10. Annual report; provisos.** Said station shall make an annual report to the Governor upon adulterated food or drug products in addition to the reports required by law which shall not exceed one hundred and fifty pages, and such annual reports shall be submitted to the General Assembly at its regular session, and said station may issue from time to time a bulletin giving the results of the inspections and of all analyses of samples taken or submitted for examination under this act, together with the names of the parties from whom the samples were taken, or where the inspections were made, and as far as possible the name of the manufacturers, the number of samples found to be adulterated, the number found not adulterated, and other information which may be of interest to the manufacturers or dealers in food or <sup>a</sup> drug products or to the consumers. Provided, however, That before such publication is made the manufacturer of the article and the dealer shall be furnished a true copy of the facts to be published regarding the article at least thirty days before the publication and hearing given the dealer and manufacturer, and any statements or explanations made by such manufacturer shall be included in the same place and along with the publication made regarding the article. And provided further, That if at the hearing of the manufacturer or dealer, as provided by section 9 hereof, said manufacturer shall produce the affidavit of a competent analytical chemist controverting the finding of said station or its director or chemist, as the case may be, and affirmatively showing that there is neither adulteration or misbranding of such article under the provisions of this act, then there shall be no publication of either the name of the manufacturer or dealer, or of the name of the brand of the article until after a trial and a verdict of guilty as herein provided. And provided further, That where prosecution is made for violation of any of the provisions of this act, no official publication shall be made of the result of the inspection and analysis until the matter has been finally adjudicated, and in case of appeal, by the court of last resort.

**SEC. 11. Cost of analysis; appropriation; expenditures.** Said Experiment Station shall receive seven dollars and fifty cents (\$7.50) for the analysis or examination of any sample of food or drug taken or submitted in accordance with

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<sup>a</sup> So in Statutes.

this act, and expenses for procuring samples of food and drugs and in making inspections into the condition of and wholesomeness and purity of the food produced, manufactured or sold in food factories, grocery stores, bakeries, slaughtering houses, dairies, milk depots or creameries, and all other places where foods are produced, prepared, stored, kept or offered for sale; for studying the problems connected with the production, preparation and sale of foods; for expert witnesses attending grand juries and courts; clerk hire and all other expenses necessary for carrying out the provisions of this act. Provided, The total expense from all sources shall not exceed in any one year thirty thousand dollars (\$30,000.00.)

The Board of Control of said Experiment Station shall furnish to the Auditor of Public Accounts an itemized statement of the expenditures of money under this act. The expenditures reported to the Auditor shall be paid by the Commonwealth to the treasurer of the Experiment Station upon the written request of the Board of Control of the said Experiment Station, and the Auditor for the payment of the same is directed to draw his warrant upon the Treasurer as in all other claims against the Commonwealth.

SEC. 12. *Filing of label, brand, etc.* When any manufacturer shall offer any article of food or drug for sale in the State he shall file with the director of the said station, when requested by him, the name of the brand, the name of the product, the place of its manufacture or preparation, and a true copy of all labeling used thereupon. Failure to so file within thirty days shall be punished as provided in section 1 of this act.

SEC. 13. *Guaranty as evidence.* In all prosecutions under this act, the courts shall admit as evidence a guaranty which has been made to the holder of the guaranty by any manufacturer or wholesaler residing in this State, to the effect that the product complained of is not adulterated or misbranded within the provisions of this act. And said guaranty, properly signed by the wholesaler, jobber or manufacturer or other party residing within this State from whom the holder of the guaranty may have purchased the article or articles complained of, and containing the full name and address of the party or parties making the sale of such article to the holder of the guaranty, and in the absence of any proof that the article or articles complained of were adulterated or misbranded after they had been received by the holder of the guaranty, shall be a bar to prosecution of the holder of such guaranty under the provisions of this act.

SEC. 14. *Repeal.* All acts or parts of acts inconsistent herewith are hereby repealed, but this said act shall not be construed to repeal Chapter 48 of the Acts of the General Assembly of 1906, entitled, "An Act to regulate the sale of concentrated commercial feeding stuffs, defining same and fixing penalties for violations thereof."

So much of this act as relates to drugs and liquors shall not take effect until on and after January 1, 1909.

Approved March 13, 1908. Acts of 1908, ch. 4, pp. 10-22.

## DAIRY PRODUCTS.

See General Food Laws.

## LIQUORS.

See General Food Laws.

## LOUISIANA.

The food and drug regulations of the Louisiana board of health have the force of law and are therefore quoted in full except when they are the same as the Federal law, regulation, or standard on a given point, in which case the appropriate section is referred to.

### REGULATIONS.

**REG. 1. *Title of the Rules and Regulations.*** The Louisiana State Board of Health hereby decrees and establishes the following Rules and Regulations governing the manufacture, sale, and inspection of foods, liquors, waters, and drugs within the State.

The Rules and Regulations of the Louisiana State Board of Health governing the manufacture, sale and inspection of foods, drugs, liquors or waters, shall be known and referred to as "The Food and Drug Regulations of the Louisiana State Board of Health."

**REG. 2. *Prohibiting adulteration or misbranding of food and drugs.*** It shall be unlawful for any person or persons to manufacture within this State any article of food, drugs, liquors or waters which is adulterated or misbranded within the meaning of these Regulations; and any person who shall violate any of the provisions of these Regulations shall be punished as provided for in Sec. 3, Act 98 of 1906.

**REG. 3. *Prohibiting importation or exportation of adulterated or misbranded food, drugs, liquors or waters.*** That the introduction into this State from any other State or Territory, or from the District of Columbia, or from any foreign country, of any article of food, drugs, liquors or waters, which is adulterated or misbranded within the meaning of these Regulations is hereby prohibited, that any person who shall receive from any State or Territory, or the District of Columbia or foreign country, and having so received, shall deliver in unbroken or broken packages, for pay or otherwise, or offer to deliver to any other person any such article so adulterated or misbranded within the meaning of these Regulations, or any person who shall sell or offer for sale, or have in his possession for sale in this State any such adulterated or misbranded foods, drugs, liquors or waters, shall be punished as provided for in Section 3, Act 98 of 1906.

Provided: That no article shall be deemed misbranded or adulterated within the provisions of these Regulations, when intended for export to any foreign country and prepared and packed according to the specifications or directions of the foreign purchaser when no substance is used in the preparation or packing thereof in conflict with the laws of the foreign country to which said article is intended to be shipped; but if said article shall be in fact sold or offered for sale for domestic use or consumption, then this provision shall not exempt said article from the operation of any of the other provisions of these Regulations.

*Broken or unbroken packages defined.* The term "Broken or unbroken package" as used in these Regulations, is the original package or part thereof, carton, case, box, barrel, bottle, phial, or other receptacle put up by the manufacturer to which the label is attached, or which may be suitable for the attachment of a label, making one complete package of the food, drug, liquor or water article.

The original package contemplated includes both the wholesale and the retail packages.

REG. 4. *State Food Commissioner, Dairy Commissioner, Analyst.* The President of the Louisiana State Board of Health shall be ex officio State Food Commissioner. The State Food Commissioner may, with the advice and consent of the Louisiana State Board of Health, appoint two or more Assistant Commissioners, each one of acknowledged standing, ability, and integrity, one of whom shall be an expert in the matter of dairy products, and one of them shall be a practical and Analytical Chemist, who shall be known as the State Analyst. The salaries of each assistant shall be fixed by the State Food Commissioner, by and with the consent of the Louisiana State Board of Health. In case of the absence or inability of the State Analyst to perform all the duties of his office or for the purpose of expediting the work of the Department, the State Food Commissioner may appoint some competent person to assist in the same temporarily.

REG. 5. *Inspectors.* The State Food Commissioner shall have authority, by and with the consent of the Louisiana State Board of Health, to appoint necessary inspectors, to assist in the work of the State Food Commissioner at such times and for such periods of time as may be required in the enforcement of the dairy, drug and food laws of the State. Such Inspectors shall have the same right of access to places to be inspected as the State Food Commissioner. The compensation of such Inspectors shall be fixed by the State Food Commissioner, by and with the consent of the Louisiana State Board of Health.

REG. 6. *Duty of State Food Commissioner.* It shall be the duty of the State Food Commissioner to enforce all the rules and regulations herein provided for or that may hereafter be enacted by this Board regarding the production, manufacture or sale of dairy products or the adulteration of any article of food or drugs, liquors or waters, and personally or through his assistants, to inspect any article of food, drugs, liquors or waters, made or offered for sale or held in possession for sale, which he may, through himself or his assistants, expect or have reason to believe to be impure, unhealthful, adulterated or misbranded, and to prosecute or cause to be prosecuted any person or persons, firm or firms, corporation or corporations, engaged in the manufacture or sale of any adulterated or misbranded article or articles of food, drugs, liquors or waters, contrary to these Regulations.

REG. 7. *Examination of foods, drugs, liquors or waters, collection of samples. Methods of analysis.* The examination of foods, drugs, liquors or waters shall be made by the State Analyst or his Assistants under the direction of the State Food Commissioner for the purpose of determining from such examinations whether such articles are adulterated or misbranded within the meaning of these regulations, and if it shall appear from any such examination that any of such specimens are adulterated or misbranded within the meaning of these Regulations, the State Food Commissioner shall cause notice thereof to be given to the party from whom such sample was obtained; any party so notified shall be given an opportunity to be heard under such other rules and regulations as may be prescribed by this Board, and if it appears that any of their rules and regulations have been violated by such party, then the State Food Commissioner shall at once certify the facts to the District Attorney of the District



wherein the offense was committed with a copy of the results of the analysis or the examination of such article duly authenticated by the Analyst or officer making such examination under the oath of such officer: after judgment of the Court, notice shall be given by publication in such manner as may be prescribed by this Board.

**REG. 8. *Collection of samples.*** Samples of broken or unbroken packages shall be collected only by Inspectors appointed by the State Food Commissioner, or by the Health, Food, or Drug Officer of the cities and towns of Louisiana. Samples may be purchased in the open market, and if in bulk, the marks, brands or tags upon the package, carton, container, wrapper or accompanying printed or written matter shall be noted. The Inspector shall also note the names of the vendor and agent through whom the sale was actually made, together with the date of purchase. The Inspector shall purchase representative samples. A sample taken from bulk goods shall be divided into three (3) parts and each part shall be labeled with the identifying marks. All samples shall be sealed by the Inspector with a seal provided for the purpose. If the package be less than four (4) pounds, or in volume less than two (2) quarts, three (3) packages of approximately the same size shall be purchased when practicable, and the marks and tags upon each noted as above. One sample shall be delivered to the party from whom purchased, one sample shall be sent to the Food Laboratory of the State Analyst, and the third sample shall be held under seal by the State Food Commissioner.

**REG. 9. *Methods of analysis.*** Unless otherwise directed by the State Board of Health the methods of analysis employed shall be those prescribed by the Association of Official Agricultural Chemists and the United States Pharmacopoeia.

**REG. 10. *Hearings.*** (A) When the examination or analysis shows that the provisions of the "Food and Drug Regulations of the Louisiana State Board of Health" have been violated, notice of the fact together with a copy of the findings shall be furnished to the party or parties from whom the sample was obtained, and a date shall be fixed at which such party or parties may be heard before the State Food Commissioner, or such other official connected with the Food and Drug Inspection service as may be commissioned by the State Food Commissioner for that purpose; the hearings shall be held at a place to be designated by the State Food Commissioner most convenient for all parties concerned. These hearings shall be private and confined to questions of fact. The parties interested therein may appear in person, or by Attorney, and may propound interrogatories, and submit oral or written evidence to show any fault or error in the findings of the Analyst or Examiner. The State Food Commissioner may order a re-examination of the samples or have new samples drawn for further examination.

When an article examined by the State Analyst is found to come in conflict with the regulations of the Louisiana State Board of Health, a written notice shall be served at once on the person or persons, or dealer or dealers, offering the same for sale, warning him or them not to sell or expose for sale such condemned article or articles.

(B) Whenever it would appear to the best interest of the public health and welfare, the Food Commissioner of the Louisiana State Board of Health is required to render such condemned articles of food, drugs, liquors or waters, unfit for consumption by man or animals.

(C) In the event that such person or persons, shall continue to violate these Regulations by selling, offering for sale, or hold in possession for sale or barter, such condemned article or articles, the State Food Commissioner shall lay

before the District Attorney of the District in which the violation occurred, the evidence of such violation, together with a copy of the analysis of the State Analyst.

(D) In the event the District Attorney should fail to promptly institute proceedings in a court of competent jurisdiction, the State Food Commissioner shall place the whole matter in the hands of the Attorney General of the State.

REG. 11. *Definition of the words foods and drugs as used herein.* (A) The term drug as used in these Regulations shall include all substances, compounds, and preparations recognized in the United States Pharmacopœia or National Formulary, for internal or external use, and any other substance or mixture of substances intended to be used for the cure, mitigation or prevention of disease of either man or other animals.

(B) The term "food" as used herein, shall include all articles intended for food, drink, confectionery, condiment, or used in the preparation thereof, whether simple, mixed, or compound.

REG. 12. *Food adulterations defined.* For the purposes of these Regulations, an article shall be deemed to be adulterated in case of foods: See Standards for Foods, Reg. No. 45; [also the Federal Food and Drugs Act, "Sec. 7. In the case of food." 1 to 6, inclusive, with the addition of the following clause after "Fifth"]: Not excluded under this provision are substances properly used in the preparation of food products for clarification or refining, and elimination in the further process of manufacture.

*Powdering, coating, and staining.* [See Federal Reg. 12.]

REG. 13. *Misbranding.* [See Federal Food and Drugs Act, sec. 8, also.]

*In case of drugs:* \* \* \*

Fourth—If the package containing it or its label shall bear any statement, design or device which shall be false or misleading in any particular.

Provided: That an article of food which does not contain any added poisonous or deleterious ingredients shall not be deemed to be adulterated or misbranded in the following cases:

First—In the case of mixture or compounds which may be now or from time to time hereafter known as articles of food, under their own distinctive names, and not an imitation of or offered for sale under the distinctive name of another article, if the name be accompanied on the same label or brand with a statement of the place where said article has been manufactured or produced.

Second—In the case of articles labeled, branded or tagged so as to plainly indicate that they are compounds, imitations, or blends, and the word "compound," "imitation," or "blend" as the case may be, is plainly stated on the package in which it is offered for sale. Provided: That the term blend, as used herein, shall be construed to mean a mixture of like substances.

REG. 14. *Label.* (a) The term "label" applies to any printed, written, pictorial, or other matter upon or attached to any package of a food or drug product, or any container thereof, including ink written, typewritten, or stencilled labels of druggists.

(b) The principal label shall consist, first—the name of the substance or product; the name of place of manufacture in the case of food compounds or mixtures; words which show that the articles are compounds, mixtures or blends; the words "compound," "mixture" or "blend," or the words designating the substances or their derivatives, and proportions required to be named in the case of drugs; and in the case of foods, the constituents are to be named in the order of their relative proportion.

All these required words shall appear upon the principal label with no intervening description or explanatory reading matter.

Second—If the name of the manufacturer and place of manufacture are given, they shall also appear upon the principal label.

Third—Elsewhere upon the principal label other matter may appear in the description of the manufacturer.

(c) The principal label on food or drugs for domestic commerce shall be printed in English (except as hereinafter provided for), with or without the foreign label in the language of the country where the food or drug product is produced or manufactured.

The size of type shall not be smaller than 8-point (brevier) caps: Provided, that in case the size of the package will not permit the use of 8-point (brevier) cap type, the size of the type may be reduced proportionately.

(d) The form, character and appearance of the labels, except as provided above, are left to the judgment of the manufacturer.

(e) Descriptive matter upon the label shall be free from any statement, design or device regarding the article or the ingredients or substances contained therein, or quality thereof or place of origin, which is false or misleading in any particular.

(f) An article containing more than one food product or active medicinal agent, is misbranded if named after a single constituent.

(g) The term "design" or "device" applies to pictorial matters of every description, and to abbreviations, characters, or signs for weights, measures or names of substances.

(h) The use of any false or misleading statement, design or device shall not be justified by any statement given as the opinion of an expert or other person, appearing on any part of the label, nor by any descriptive matter explaining the use of the false or misleading statement, design or device.

*Name and address of manufacturer.* [See Federal Reg. 18-20 and secs. (b) and (c) of Reg. 21.]

*Distinctive name.* [See Federal Reg. 20.]

(u) A color or flavor cannot be employed to imitate any natural product or any other product of recognized name and quality, except as especially provided for in Regulations 38 and 45—sections covering Root Beer, Candy and Confectionery.

*Imitation.* [See Federal Regs. 21-22.]

REG. 15. *Mixtures or compounds, with distinctive names.* [See Federal Reg. 27.]

REG. 16. *Proper branding not a complete guarantee.* [See Federal Reg. 23.]

REG. 17. *Incompleteness of branding.* [See Federal Reg. 24.]

REG. 18. *Substitution.* [See Federal Reg. 25.]

REG. 19. *Waste material.* [See Federal Reg. 26.]

REG. 20. [*Relates to drugs.*]

REG. 21. *Inspection of raw materials.* The State Food Commissioner, when he deems it necessary, shall examine or cause to be examined, the raw materials used in the manufacture of food and drug products, and determine whether any filthy, decomposed, or putrid substance is used in their preparation.

REG. 22. *Working formula required.* The State Food Commissioner shall have furnished him on demand a certified copy of the working formula used in the manufacture of any compound of drugs, foods, liquors or waters, when in his judgment the safety of the public health and the enforcement of these Regulations demand it. It being well understood that said certified copy be and remain the property of the manufacturer furnishing the same; and shall be regarded strictly as a confidential communication.

REG. 23-25. [*Relate to drugs.*]

REG. 26. *Substances named in drugs or foods.* [See Federal Reg. 28, a, b, c, and f.]

The following articles shall be included in the above list and become subject to the rules and regulations governing it on and after October 1, 1908:

- Nux vomica, its active principles and preparations.
- Gelsemium, its active principles and preparations.
- Physostigma, its active principles and preparations.
- Belladonna, its active principles and preparations.
- Scopola, its active principles and preparations.
- Hyoscyamus, its active principles and preparations.
- Stramonium, its active principles and preparations.
- Veratrum viride, its active principles and preparations.
- Staphisagria, its active principles and preparations.
- Aconite, its active principles and preparations.
- Colchicum, its active principles and preparations.
- Pilocarpus, its active principles and preparations.
- Pelletierine, its active principles and preparations.
- Conium, its active principles and preparations.
- Scoparius, its active principles and preparations.
- Digitalis, its active principles and preparations.
- Convallaria, its active principles and preparations.
- Strophanthus, its active principles and preparations.
- Male fern, its active principles and preparations.
- Santonin, its active principles and preparations.
- Ergot, its active principles and preparations.
- Gossypii cortex, its active principles and preparations.
- Elaterium, its active principles and preparations.
- Croton oil, its active principles and preparations.
- Cantharides, its active principles and preparations.
- Antimony, its compounds and preparations.
- Mercury, its compounds and preparations, except calomel and mercury in metallic state.
- Arsenic, its compounds and preparations.
- Potassium cyanide and hydrocyanic acid.
- Carbolic acid.

Any synthetical compound having the property of relieving pain, producing sleep, or reducing temperature.

REG. 27. [*Relates to drugs.*]

REG. 28. *Statement of weight or measure.* [See Federal Reg. 29, also the following]:

(c) In the case of alcohol the expression "quantity" or "proportion" shall mean the average percentage by volume in the finished product.

(d) In the case of the other ingredients required to be named upon the label, the expression "quantity" or "proportion" shall mean grains or minims per ounce or fluid ounce, per unit, per tablet, pill, etc., and also, if desired, the metric equivalents therefor, or milligrams per gram or per cubic centimeter, or grams or cubic centimeters per kilogram or per litre.

REG. 29. *Imported food and drug products.* Food products intended for export containing added substances not permitted in foods intended for consumption in this State, but in accordance with the directions of the foreign purchaser, must be kept separate and labeled to indicate that they are for export.

If these products are not exported, they shall not be allowed to be sold, bartered or given away for consumption in this State.

Meat and meat food products as well as all other food and drug products of a kind forbidden entry into or forbidden to be sold, or restricted in sale in the country in which made or from which exported, must not be sold, bartered or given away in this State.

REG. 30. *Denaturing.* [See Federal Reg. 34.]

REG. 31. *Instruction to inspectors.* In sending in samples for analysis to this Department of any manufactured product, the following information must accompany each sample, to-wit:

(a) Name and location of manufacturer or dealer. If bought of jobbers, the firm name and location plainly written in ink.

Brand or name of article, any representation by seller as to quality or character of goods.

To enable correct analysis to be made, not less than the following quantities of each article should be sent:

Bread, not less than 16 ounces.

Butter, not less than 8 ounces.

Baking powder, not less than 1 small can.

Beer, not less than 1 pint.

Buckwheat flour, not less than 8 ounces.

Cheese, not less than 6 ounces.

Candy, not less than 8 ounces.

Cocoa and

Chocolate, in small original package.

Cream of tartar, not less than 1 ounce.

Cream, not less than 4 ounces.

Extracts, not less than 2 ounces.

Honey, not less than 8 ounces.

Jellies, not less than  $\frac{1}{2}$  lb., or small original package.

Jams, not less than  $\frac{1}{2}$  lb., or small original package.

Liquor, not less than 1 pint.

Lard, not less than 4 ounces.

Maple sugar, not less than 1 pound.

Molasses and syrups, not less than 1 pint.

Milk, not less than 4 ounces.

Olive oil, not less than 4 ounces.

Preserves, not less than  $\frac{1}{2}$  lb., or small original package.

Spices, not less than 4 ounces.

Sugar, not less than 8 ounces.

Vinegar, not less than 1 pint.

Wine, not less than 1 pint.

Goods should be procured in original package when put up in packages containing not more than two pounds solid or one-half gallon liquid measure.

REG. 32. *Baking powder.* No person in this State shall make or manufacture baking powder or any other mixture or compound intended for use as baking powder, or sell, exchange, deliver, or offer for sale or exchange, such baking powder, or any mixture or compound intended for use as baking powder, unless its composition be distinctly shown by a label on the outside and face of which is printed with black ink in a legible type, with roman letters not less than 8-point—brevier—cap on a white or light black background, the manufacturer's name and the place of manufacture and in a conspicuous place on the face of the label of such package of baking powder and with letters similar in size, the name of the acid ingredient together with a list of all the ingredients entering into its composition.

Provided, the use of any substance deemed poisonous or injurious is hereby prohibited and the use thereof in the manufacture of baking powder is hereby declared unlawful. Baking powders must yield at least 8 per cent available carbon dioxide. The use of argolite, terra alba and all other mineral fillers is prohibited.

Baking powders must have specific name of the powder on the label and no ingredient can be named as a component of the powder not found in the article.

REG. 33. *To regulate the manufacture and sale of substitutes of butter.*

(a) That for the purpose of these regulations every article, substitute or compound of any other than that which is produced from pure milk or cream, therefrom made in a semblance of butter and designated to be used as a substitute for butter made from pure milk or its cream, is hereby declared to be imitation butter.

Provided: That the use of salt and harmless coloring matter for coloring the product of pure milk or cream shall not be construed to render such product an imitation.

(b) No person shall coat, powder or color with annato or any injurious coloring matter whatever, any substance designed as a substitute for butter whereby such substitute or product so colored or compounded shall be made to resemble butter, the product of the dairy and sold as such. No person shall combine any animal fat with butter and sell the same for consumption.

Provided: nothing in these regulations shall be construed to prohibit the use of salt, rennet and harmless coloring matter for coloring the product of pure milk or cream from the same.

(c) No person shall produce or manufacture any substance or semblance in imitation of natural butter, nor sell or keep for sale, barter or give away, nor offer for sale any imitation butter made, manufactured, compounded or produced in violation of this Regulation whether such imitation butter shall be made or produced in this State or elsewhere; this Regulation shall not be construed to prohibit the manufacture and sale under the Regulations hereinafter provided of substances designed to be used as a substitute for butter, and not manufactured or colored as herein provided.

(d) Every person who lawfully manufactures any substance designed to be used as a substitute for butter, shall mark by branding, stamping or stenciling upon the top side of each box, tub, firkin, or other package in which such article shall be kept, and in which it shall be removed from the place where it is produced, in a clear and durable manner in the English language the word "Oleomargarine" or the word "Butterine" or the words "Substitute for Butter" or the words "Imitation Butter" in printed letters, in plain roman type; each of which shall not be less than three-fourths of an inch in length.

(e) It shall be unlawful to sell or offer for sale, barter or give away, any imitation butter without informing the purchaser thereof, or the person or persons to whom the same is offered for sale, that the substance sold or offered for sale is imitation butter.

(f) No person by himself or with others shall ship, consign or forward, by any common carrier whether public or private, any substance designed to be used as a substitute for butter, unless it shall be marked or branded on each tub, box, firkin, jar or other package containing the same, as provided in this Regulation, and unless it be consigned by the carriers and receipted for by its true name.

(g) No person shall have in his possession or under his control, any substance designed to be used as a substitute for butter unless the tub, firkin, jar, box or other package containing the same be clearly and durably marked as provided in this Regulation.

Every person who shall have possession or control of any imitation butter for the purpose of selling, bartering, or giving away the same, which is not marked as required by the provisions of this Regulation, shall be presumed to have known during the time of such possession or control, the true character and name as fixed by this Regulation of such product.

(h) Whoever shall have possession or control of any imitation butter or any substance designed to be used as a substitute for butter contrary to the provisions of this Regulation, for the purpose of selling the same or offering the same for sale, barter or give away, shall be held to have possession of such property with intent to use it in violation of this Regulation.

(i) Whoever shall deface, erase or remove any mark provided by this Regulation, with intent to mislead, deceive or violate any of the provisions of this Regulation, shall be held liable to the penalties herein provided for a violation of any of these Regulations.

(j) That no person, firm, corporation, agent, or employe shall manufacture, sell, offer or expose for sale in this State, any butter that is produced by taking original packing stock butter, or other butter, or both, and melting the same, so that the butter fat can be drawn off or extracted, then mixing the said butter fat with skimmed milk, or milk, or cream, or other milk product and re-churning or reworking the said mixture, or that produced by any process that is commonly known as boiled, process, or renovated butter unless the same is branded or marked as provided in this Regulation.

(k) No person, firm, corporation, agent or employe shall sell, offer or expose for sale, barter, or give away, or deliver to purchaser any boiled, process, or renovated butter unless the words: "Renovated Butter" shall be plainly branded with gothic or bold faced letters at least three-fourths of an inch in length on the top and sides of each tub, or box, or pail, or other kind of a case or package, or on the wrapper of prints or rolls in which it is put. If such butter is exposed for sale uncovered or not in a case or package, a placard containing the label so printed, shall be attached to them in such a manner as to be easily seen and read by the purchaser. The branding or marking of all packages shall be in the English language, in a conspicuous place so as to be easily seen and read by the purchaser.

(l) Every hotel, restaurant or boarding house, using any imitation, processed, or renovated butter, must state the true nature of the imitation or processed butter used on the bill of fare or on a placard conspicuously placed, and printed in bold type and in the English language.

(m) The State Food Commissioner and his assistants, experts, chemists or agents shall have access and ingress to all places of business, factories, stores, and buildings used for the manufacture or sale of butter. They shall have power and authority to open any tub, box, pail or other kind of case or package containing any butter that may be manufactured, sold or exposed for sale.

**REG. 34. Candy, confectionery, cocoa, chocolate.** (a) In the case of confectionery:

It shall be considered adulterated if it contains terra alba, barytes talc, chrome yellow, or other mineral substance or poisonous color or flavor or other ingredient deleterious or detrimental to health, or any vinous, malt or spiritous liquor or compound or narcotic drug.

Candy must not be wrapped in tin foil in direct contact with the candy.

**REG. 35. Canned goods.** No packer or dealer in preserved or canned fruits and vegetables or other articles of food shall sell or offer for sale such canned or preserved fruits and vegetables or other articles unless they shall be entirely free from substances or ingredients deleterious to health, or use dyes

or coloring matter whereby their true character would be disguised or inferiority concealed.

The addition of sugar to a substance not naturally sweet, converting it into a substance which might seem naturally sweet is permitted, if the label plainly indicates that sweetening material has been added.

All soaked or bleached goods or goods put up from products dried before canning shall be plainly marked, branded, stamped or labeled as such with the words "Soaked" or "Bleached goods" in letters of equal size to that of the name of the product and bearing the name of the article and name and address of the packer or dealer who sells same.

REG. 36. *Cold storage.* The sale of milk or cream that has been kept over 24 hours in cold storage, the sale of fish that has been kept over 24 hours in cold storage, the sale of meat that has been kept over three weeks in cold storage is prohibited unless the facts in regard to the same are certified to the purchaser.

REG. 37. *Coffee.* Coffee must be true to name.

It must not be coated, colored or polished when such coating, coloring or polishing injures the coffee, or conceals some damage or inferiority.

Imitations containing no coffee cannot be sold as coffee compounds, but must be sold as imitation coffee, with the statement that they contain no coffee.

Compounds of coffee and chicory, or of coffee and any other harmless substitute allied to it, either in flavor or strength and not used simply as an adulterant may be sold when labeled Coffee Compound and such compound must state on face of label the names of the ingredients used in making the compound in the order of their relative proportions, in type of equal size and prominence.

REG. 38. *Fruit syrup, soda water syrup, pop, soft drinks, etc.* Drinks containing less than two per cent of alcohol, fruit syrups, soda water syrups, pops, soft drinks, etc., shall not contain any saccharin, salicylic, or boric acid, their derivatives, or any harmful coloring matter or preservative. All drinks containing less than two per cent of alcohol, fruit syrups, soda water syrups, pops, soft drinks, etc., made from any substance except the natural extract of fruit, or flavored or colored with synthetical products, must have the word "artificial" printed on the label of the package in the same size, style and color of letter and background as the name of the article, and in such a manner as to show that they have no relation whatsoever to the fruit which they imitate. All soda fountains or places where the above mentioned "artificial" articles are sold or served, shall have printed on a placard the words, "artificial drinks" and hung in front of the fountain or in a conspicuous place.

See Reg. 45 for list of permitted "Coal Tar Dyes."

The use of 1-10 of one per cent of benzoate of soda, is permitted in natural or artificial fruit syrups.

The use of saccharin in any food product is prohibited.

The terms "Artificial" and "Imitation" may be used synonymously.

REG. 39. *Honey.* No person, firm or corporation shall offer for sale, or possess with intent to sell, barter or give away, honey manufactured from or mixed with glucose, sugar, or syrup of any kind, or any substance not the legitimate product of the honey bee, unless the package containing same is so marked and represented as such and bearing a label upon the package printed in heavy Gothic capitals, 18 point, with the name of the person manufacturing or mixing the same, and the name of the substance or material from which it is compounded.

REG. 40. *Ice.* No person, firm or corporation shall manufacture, sell or deliver for food or drink purposes, any ice natural or manufactured, containing decomposed, putrid, infected, tainted, or rotten animal or vegetable substances,



or any ingredient injurious to health. Nor ice made from water of a lower standard of purity than that required for potable water by the State Board of Health, as indicated in its Standards and definitions of Food Products.

**REG. 41. Lard.** (a) No person, firm or corporation shall manufacture or sell lard to which has been added beef or mutton fat, stearine, cotton seed oil, or other substitute for swine fat, unless the container is plainly marked "adulterated" or "Lard Compound" in bold letters and the quantity and name of the adulterant is made part of the label.

(b) Lard, lard compounds, or lard substitutes, containing more than one (1) per cent of water, shall be considered adulterated.

**REG. 42. Adulteration of wines.** (a) All wine containing alcohol, except such as have been produced by natural fermentation of pure undried fruit juices, or combined with distilled spirits, whether denominated wines or by any other name, which may be used as a beverage or combined with other liquors intended for use, and all compounds of the same with pure wine, and all preserved fruit juices compounded with substances not produced from undried fruit intended for use as a beverage or for use in the fermentation or preparation of liquors intended for such use, and all wines, imitations of wines, or other beverages produced from fruit which shall contain alum, baryta, lime, carbonate of soda, salts of lead, salicylic acid, or any other antiseptic or coloring matter not produced from undried fruit, or which contains artificial flavoring, essence of ether or any other foreign substance injurious to health, shall be known as, or deemed to be adulterated wine, and shall not be sold, offered for sale, barter or give away or manufactured with intent to sell, barter or give away, within the State.

**REG. 43. Sugars, syrups and molasses must conform to the standards laid down.** (A) It shall be unlawful for any person or persons, firm or corporation or agent thereof, to sell, advertise, or offer for sale, barter or give away within the limits of this State, any compound or mixed syrup, unless at the time of sale, the names of the ingredients in the order of their relative proportion of such mixture or compound are clearly stamped or labeled on the bottle, can, case, barrel or other receptacle containing such syrup.

The term "Mixture or Compound" as used in this Regulation is understood to apply to all mixtures or compounds of two or more ingredients differing in their nature or quality such as sugar cane syrup, sorghum cane syrup, maple syrup, molasses or glucose (corn syrup.)

Finished syrups or molasses, containing zinc or tin compounds, will be condemned as food products.

All packages of mixed or compound syrups in barrels, cans, bottles or other containers shall be labeled with the name of the manufacturer and the place of manufacture.

(B) In the manufacture of syrups and molasses the use of sulphur as a clarifying agent is permissible, Provided: the residual sulphur does not exceed 1-10 of one per cent.

**REG. 44. Principles on which the standards are based.** [See Cir. 19, Office of the Secretary, Federal Standards. These were adopted in toto and only the standards adopted in addition to those promulgated by the Secretary of Agriculture (Cir. 19) are here given.]

**REG. 45. Food standards.** [See also Cir. 19, Office of the Secretary, Federal Standards.]

*c. Meat extracts, meat peptones, gelatine, etc.* 1. *Meat extract* is the product obtained by extracting fresh meat with boiling water and concentrating the liquid portion by evaporation after the removal of fat, and contains not less than seventy-five (75) per cent of total solids, of which not over twenty-seven

(27) per cent is ash, and not over twelve (12) per cent is sodium chlorid (calculated from the total chlorin present), not over six-tenths (0.6) per cent is fat, and not less than eight (8) per cent is nitrogen. The nitrogenous compounds contain not less than forty (40) per cent of meat bases and not less than ten (10) per cent of kreatin and kreatinin.

2. *Fluid meat extract* is identical with meat extract except that it is concentrated to a lower degree and contains not more than seventy-five (75) and not less than (50) per cent of total solids.

3. *Bone extract* is the product obtained by extracting fresh trimmed bones with boiling water and concentrating the liquid portion by evaporation after removal of fat, and contains not less than seventy-five (75) per cent of total solids.

4. *Fluid bone extract* is identical with bone extract except that it is concentrated to a lower degree and contains not more than seventy-five (75) and not less than fifty (50) per cent of total solids.

5. *Meat juice* is the fluid portion of muscle fibre, obtained by pressure or otherwise, and may be concentrated by evaporation at a temperature below the coagulating point of the soluble proteids. The solids contain not more than fifteen (15) per cent of ash not more than two and five-tenths (2.5) per cent of sodium chlorid (calculated from the total chlorin present) not more than four (4) nor less than two (2) per cent of phosphoric acid ( $P_2O_5$ ), and not less than twelve (12) per cent of nitrogen. The nitrogenous bodies contain not less than thirty-five (35) per cent of coagulable proteids and not more than forty (40) per cent of meat bases.

6. *Peptones* are products prepared by the digestion of proteid material by means of enzymes or otherwise, and contain not less than ninety (90) per cent of proteoses and peptones.

7. *Gelatin (edible gelatine)* is the purified, dried, inodorous product of the hydrolysis, by treatment with boiling water, of certain tissues, as skin, ligaments, and bones, from sound animals, and contains not more than fifteen (15) per cent and not less than two (2) per cent of nitrogen.

*Sauces.* Must be made from sound and wholesome materials. The use of a filler is prohibited. Must contain no sweetening material other than pure sugar. Must not contain added salicylic acid, benzoic acid, saccharin, boric acid, formaldehyde, chemical preservatives or their derivatives or coloring matter. If distilled vinegar is used, it shall be so stated on the label.

*Pickles.* Must be made from sound and wholesome materials. Must contain no sweetening agent other than pure sugar. Must not contain added salicylic acid, benzoic acid, saccharin, boric acid, formaldehyde, chemical preservative or their derivatives, copper salts, alum, iron salts or coloring matter. If distilled vinegar is used it must be so stated on the label.

*Red pepper sauce.* Must be made from sound, ripe, wholesome *Red pepper*, and must contain no added filling; must not contain added salicylic acid, benzoic acid, saccharin, boric acid, formaldehyde, chemical preservatives or their derivatives or coloring matter. If distilled vinegar is used it must be so stated on the label.

*Catsup.* Must be made from ripe, wholesome and sound vegetable materials. The use of a filler of starch or other matter is prohibited. Must not contain added salicylic acid, benzoic acid, saccharin, boric acid, formaldehyde, or their derivatives, nor any added chemical preservative or coloring matter. If distilled vinegar is used it shall be so stated on the label.

**F. Beverages.** *a. Fruit juices—fresh, sweet, and fermented. Fresh fruit juices.* 1. *Fresh fruit juices* are the clean, unfermented liquid products ob-

tained by the pressing of fresh, ripe fruits, and correspond in name to the fruits from which they are obtained.

2. *Apple juice, apple must, sweet cider*, is the fresh fruit juice obtained from apples, the fruit of *Pyrus malus*, has a specific gravity (20° C.) not less than 1.0415 nor greater than 1.0690; and contains in one hundred (100) cubic centimetres (20° C.) not less than six (6) grams, and not more than twenty (20) grams of total sugars, in terms of reducing sugars, not less than twenty-four (24) centigrams nor more than sixty (60) centigrams of apple ash, which contains not less than fifty (50) per cent of potassium carbonate.

3. *Grape juice, grape must*, is the fresh fruit juice obtained from grapes (*Vitis* species), has a specific gravity (20° C.) not less than 1.0400 and not exceeding 1.1240; and contains in one hundred (100) cubic centimetres (20° C.), not less than seven (7) grams nor more than twenty-eight (28) grams of total sugars, in terms of reducing sugars, not less than twenty (20) centigrams and not more than fifty-five (55) centigrams of grape ash, and not less than fifteen (15) milligrams nor more than seventy (70) milligrams of phosphoric acid ( $P_2O_5$ ).

4. *Lemon juice* is the fresh fruit juice obtained from lemon, the fruit of *Citrus limonum* Blisso, has a specific gravity (20° C.) not less than 1.030 and not greater than 1.040; and contains not less than ten (10) per cent of solids, and not less than seven (7) per cent of citric acid.

5. *Pear juice, pear must, sweet perry*, is the fresh fruit juice obtained from pears, the fruit of *Pyrus communis* or *P. sinensis*.

*Sterilized fruit juices.* 1. *Sterilized fruit juices* are the products obtained by heating fresh fruit juices sufficiently to kill all the organisms present, and correspond in name to the fruits from which they are obtained.

*Concentrated fruit juices.* 1. *Concentrated fruit juices* are clean, sound fruit juices from which a considerable portion of the water has been evaporated, and correspond in name to the fruits from which they are obtained.

*Sweet fruit juices, sweetened fruit juices, fruit sirups.* 1. *Sweet fruit juices, sweetened fruit juices, fruit sirups*, are the products obtained by adding sugar (sucrose) to fresh fruit juices, and correspond in name to the fruit from which they are obtained.

2. *Sterilized fruit sirups* are the products obtained by the addition of sugar (sucrose) to fresh fruit juices and heating them sufficiently to kill all organisms present, and correspond in name to the fruits from which they are obtained.

9. *Cider, hard cider*, is the product made by the normal alcoholic fermentation of apple juice, and the usual cellar treatment, and contains not more than seven (7) per cent by volume of alcohol, and, in one hundred (100) cubic centimetres of the cider, not less than two (2) grams nor more than twelve (12) grams of solids, not more than eight (8) grams of sugars, in terms of reducing sugars, and not less than twenty (20) centigrams nor more than forty (40) centigrams of cider ash.

10. *Sparkling cider, champagne cider*, is cider in which the after-part of the fermentation is completed in closed containers, with or without the addition of cider or sugar liquor, and contains in one hundred (100) cubic centimetres, not less than twenty (20) centigrams of cider ash.

b. *Mead, root beer, etc. Mead.* The materials used shall be pure and wholesome according to the standards set forth in these regulations. The water used shall be potable; shall not contain added salicylic acid, benzoic acid, saccharin, boric acid, formaldehyde, or their derivatives, nor any added chemical preservative or coloring matter.

*Root beer* shall be manufactured from roots, bark, leaves, berries, herbs, or the oils extracted therefrom, caramel, or other harmless ingredients; shall not contain added salicylic acid, saccharin, boric acid, formaldehyde, or their derivatives, or added chemical preservatives or coloring matter.

*c. Malt liquors.* 1. *Malt liquor* is a beverage made by the alcoholic fermentation of an infusion in potable water, of barley-malt and hops, with or without malted cereals.

2. *Beer* is a malt liquor produced by bottom fermentation, and contains in one hundred (100) cubic centimetres, at (20° C.) not less than five (5) grams of extractive matter, and sixteen one-hundredths (.16) gram of ash, chiefly potassium phosphate, and not less than two and twenty-five one hundredths (2.25) grams of alcohol.

3. *Lager beer, stored beer,* is beer which has been stored in casks for a period of at least three months, and contains in one hundred (100) cubic centimetres (at 20° C.) not less than five (5) grams of extractive matter, and sixteen one-hundredths (.16) gram of ash, chiefly potassium phosphate, and not less than two and fifty one-hundredths (2.50) grams of alcohol.

4. *Malt beer* is beer made of an infusion in potable water, of barley malt, and hops, and containing in one hundred (100) cubic centimetres (at 20° C.) not less than five (5) grams of extractive matter, nor less than two-tenths (.2) gram of ash, chiefly potassium phosphate, nor less than two and twenty-five one-hundredths (2.25) grams of alcohol, nor less than four-tenths (.4) gram of crude protein (nitrogen  $\times$  6.25).

5. *Ale* is a malt liquor produced by top fermentation and contains in one hundred (100) cubic centimetres (at 20° C.) not less than two and seventy-five one hundredths (2.75) grams of alcohol, nor less than five (5) grams of extract.

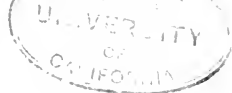
6. *Porter and stout* are varieties of ale colored by the addition of highly roasted malt to the infusion.

*d. Spirituous liquors.* 1. *Distilled spirits* is the distillate obtained from a fermented mash of cereals, molasses, sugars, fruits, or other starch- or sugar bearing substances, and contains all the condensed products of the fermentation volatile at the usual temperature of distillation.

2. *Rectified spirits* is distilled spirit which at the time of, or subsequent to distillation is subjected to a rectifying process by means of which a part of the volatile products of the distillation is separated from the ethyl alcohol therein.

3. *Alcohol, cologne spirit, neutral spirit, velvet spirit, or silent spirit* is distilled spirit from which all, or nearly all, its constituents are separated, except ethyl alcohol and water, and contains not less than ninety-four and nine-tenths (94.9) per cent (189.8 proof) by volume of ethyl alcohol.

4. *New whiskey* is the distilled spirits from the properly fermented mash of malt cereals, or cereals the starch of which has been hydrolized by malt, is of an alcoholic strength corresponding to the excise laws of the various countries in which it is made, and contains not less than one hundred and twenty-five (125) nor more than three hundred and fifty (350) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than ninety (90) nor more than two hundred and twenty-five (225) grams of fusel oil (higher alcohols as amylic), not more than twenty (20) grams of aldehydes, not less than fifteen (15) nor more than one hundred (100) grams of ethers (as acetic ether), not less than two (2) nor more than twenty-five (25) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).



5. *Whiskey (potable whiskey)* is new whiskey which has been stored in wood for not less than four (4) years and mixed only with pure water at the time of its preparation for consumption, and contains unless otherwise prescribed by law, not less than forty-five (45) per cent of ethyl alcohol by volume, and the relative quantities of secondary products to ethyl alcohol corresponding to the varieties of whiskey under six (6) to fifteen (15), inclusive.

6. *Rye whiskey* is whiskey in the manufacture of which rye is the principal cereal used, and contains not less than two hundred (200) nor more than five hundred (500) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than one hundred (100) nor more than two hundred and fifty (250) grams of fusel oil (higher alcohols as amylic), not more than twenty-five grams of aldehydes, not less than forty (40) nor more than one hundred and fifty (150) grams of ethers (as acetic ether), not less than thirty (30) nor more than eighty-five (85) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

7. *Bourbon whiskey* is whiskey in which Indian corn (maize) is the principal cereal used, and contains not less than two hundred (200) nor more than five hundred (500) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than one hundred (100) nor more than two hundred and fifty (250) grams of fusel oil (higher alcohols and amylic), not more than twenty-five (25) grams of aldehydes, not less than forty (40) nor more than one hundred and fifty (150) grams of ethers (as acetic ether), not less than thirty (30) nor more than eighty-five (85) grams of volatile acids (as acetic) to one hundred litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

8. *Corn whiskey* is whiskey made from maize (Indian corn), the starch of which has been hydrolized by malting or by the action of barley malt, and contains the proportions of the various ingredients specified for bourbon whiskey.

9. *Arrack* is distilled spirit made from rice.

10. *Blended whiskey* is a mixture of two or more whiskeys, and contains the relative quantities of secondary products to ethyl alcohol of the varieties of whiskey forming the blend.

11. *Rectified new whiskey* is new whiskey deprived of a part of its secondary volatile products, and contains not less than sixty (60) grams of the secondary volatile products of distillation congeneric with ethyl alcohol, not less than forty (40) grams of fusel oil (higher alcohol as amylic) not more than eight (8) grams of aldehydes, not less than five (5) grams of ethers (as acetic ether), not less than one (1) gram of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent of ethyl alcohol by volume).

12. *Rectified whiskey* is rectified new whiskey stored in wood not less than three (3) years, except where otherwise prescribed by law, and contains not less than one hundred (100) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than fifty (50) grams of fusel oil (higher alcohols as amylic), not more than ten (10) grams of aldehydes, not less than twenty (20) grams of ethers (as acetic ether), not less than fifteen (15) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

13. *Scotch new whiskey* is whiskey made in Scotland solely from barley malt in the drying of which over burning peat a smoky or peaty flavor is imparted to the product, and contains not less than one hundred and twenty-five (125) nor more than three hundred and fifty (350) grams of the secondary product of distillation congeneric with ethyl alcohol, not less than ninety (90) nor more

than two hundred and twenty-five (225) grams of fusel oil (higher alcohols as amylic), not more than twenty (20) grams of aldehydes, not less than fifteen (15) nor more than one hundred (100) grams of ethers (as acetic ether), not less than two (2) nor more than twenty-five (25) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

14. *Scotch whiskey* is Scotch new whiskey which has been stored in wood for not less than four years and mixed only with pure water at the time of its preparation for consumption, and contains not less than one hundred and fifty (150) nor more than four hundred and fifty (450) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than one hundred (100) nor more than two hundred and fifty (250) grams of fusel oil (higher alcohols as amylic) not more than twenty-five (25) grams of aldehyde, not less than twenty-five (25) nor more than one hundred and twenty-five (125) grams of ethers (as acetic ether), not less than ten (10) nor more than forty (40) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

15. *Irish new whiskey* is whiskey made in Ireland either from barley malt, or malt and unmalted barley, or other cereals, and contains not less than one hundred and twenty-five (125) nor more than three hundred and fifty (350) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than ninety (90) nor more than two hundred and twenty-five (225) grams of fusel oil (higher alcohols as amylic), not more than twenty (20) grams of aldehydes, not less than fifteen nor more than one hundred (100) grams of ethers (as acetic ether), not less than two (2) nor more than twenty-five (25) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

16. *Irish whiskey* is Irish new whiskey which has been stored in wood for not less than four years and mixed only with pure water at the time of its preparation for consumption, and contains not less than one hundred and fifty (150) nor more than four hundred and fifty (450) grams of the secondary products of distillation congeneric with ethyl alcohol not less than one hundred (100) nor more than two hundred and fifty (250) grams of fusel oil (higher alcohols as amylic), not more than twenty-five grams of aldehydes, not less than twenty-five (25) nor more than one hundred and twenty-five (125) grams of ethers (as acetic ether), not less than ten (10) nor more than forty (40) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

17. *New rum* is distilled spirits made from the fermented juice of the sugar cane, the massecuite made therefrom, molasses from the massecuite or any intermediate product save sugar, and contains not less than one hundred and twenty-five (125) nor more than three hundred and fifty (350) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than sixty (60) nor more than one hundred and fifty (150) grams of fusel oil (higher alcohols as amylic) not more than thirty (30) grams of aldehydes, not less than thirty (30) nor more than one hundred (100) grams of ethers (as acetic ether), not less than twenty (20) nor more than (50) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

18. *Rum* is new rum stored not less than four (4) years in wood, and contains not less than one hundred and seventy-five (175) nor more than five hundred (500) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than eighty (80) nor more than two hundred (200) grams of

fusel oil (higher alcohols as amylic), not more than forty (40) grams of aldehydes, not less than fifty (50) nor more than one hundred and fifty (150) grams of ethers (as acetic ether) not less than thirty-five (35) nor more than one hundred (100) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

19. *New brandy* is a distilled spirit made from sound potable wine, and contains not less than one hundred and twenty-five (125) nor more than three hundred and fifty (350) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than seventy (70) nor more than one hundred and fifty (150) grams of fusel oil (higher alcohols as amylic), nor more than twenty (20) grams of aldehydes, not less than thirty (30) nor more than one hundred (100) grams of ethers (as acetic ether), not less than five (5) nor more than twenty (20) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

20. *Brandy* is new brandy stored in wood for not less than four (4) years, and contains not less than one hundred and fifty (150) nor more than five hundred (500) grams of the secondary products of distillation congeneric with ethyl alcohol, not less than eighty (80) nor more than two hundred (200) grams of fusel oil (higher alcohols as amylic), not more than thirty (30) grams of aldehydes, not less than thirty-five (35) nor more than one hundred and fifty (150) grams of ethers (as acetic ether), not less than thirty (30) nor more than one hundred (100) grams of volatile acids (as acetic) to one hundred (100) litres of proof ethyl alcohol (50 per cent ethyl alcohol by volume).

21. *Cognac* is brandy prepared in the departments of the Charente, France, from pure, sound wine produced in those departments.

*e. Carbonated waters. Water—Potable—*Water to be potable must be suitable to all forms of domestic use; must possess no objectionable smell or taste; must be free from animal, especially human refuse material; must be free from vegetable material in a state of active decomposition; must be free from pathogenic bacteria; must be free from such an amount of suspended material of whatever character as would make it unsightly in appearance and unsuited to the ordinary industrial uses of a community.

*Carbonated waters* are waters charged with carbonic acid gas, and may be naturally carbonated or artificially carbonated. Label must state how carbonated, and if the source of the water is given thereon, the water must be true to its label. All carbonated waters must be wholesome and potable.

*Spring and well waters* are waters derived from springs or wells; they must be potable and wholesome; they may or may not be medicinal; and must come from the well or spring indicated on the label and no other. The standard for any spring or well water will be the water itself, sample being taken at its source by a representative of this Board.

*Artificial mineral waters* must be so labeled, and the water used in their manufacture must be wholesome and potable. All waters must be true to label, and if an analysis is published as an advertisement, or is placed on the label, the water must conform thereto.

#### G. Vinegar. \* \* \*

6. *Spirit vinegar, distilled vinegar, grain vinegar*, is the product made by the acetous fermentations of dilute distilled alcohol, and contains, in one hundred (100) cubic centimetres (20° C.), not less than four (4) grams of acetic acid, and shall be free from coloring matter, added during or after distillation, and from color other than that imparted to it by distillation.

*Bread and yeast.* Bread must be made of pure and wholesome materials as provided for in these regulations, must not contain adulterants, alums, or cop-

per salts, should not contain more than forty (40) per cent of water nor have an acidity in ten (10) grams of fresh bread requiring more than 10 c. c. of 1-10 normal sodium hydroxide solution to neutralize it.

Compressed Yeast should be used when fresh. Such yeast should have a creamy white color, uniform throughout, should possess a fine even texture, should be moist without being slimy, should not have a "cheesy" odor, such odor indicating decomposition as does a dark streaked color.

#### IV. PRESERVATIVES AND COLORING MATTERS.

*Standard preservatives* are salt, sugar, vinegar, spices, and their essential oils, wood smoke, edible oils and fats, and alcohol.

The use in food products, of any other preservatives or antiseptics, or of any substance which preserves or enhances the natural color of a food product, or of a coloring matter is prohibited except as provided for in these Regulations.

\* \* \*

See Reg. 14, Section (u) [and F. I. D. 76 as to permitted coal tar dyes.]

REG. 46. *Taking orders deemed a sale. Taking orders for same.* The taking of orders or the making of agreements or contracts by any person, firm or corporation, or by any agent or representative thereof, for the future delivery of any of the articles, products, goods, wares, or merchandise embraced within the provisions of these Regulations, shall be deemed a sale within the meaning of these Regulations.

REG. 47. *Person defined.* The word "person," as used in these regulations shall be construed to import both the plural and the singular, as the case demands, and shall include corporations, companies, societies, and associations, when construing and enforcing the provisions of these regulations the act, omission or failure of any officer, agent or other person acting for or employed by any corporation within the scope of his employment or office, shall in every case be also deemed to be the act, omission, or failure of such corporation, company, society, or association, as well as that of the person.

REG. 48. *Penalty.* Any person convicted of violating any of the provisions of the foregoing Regulations wherein penalty is not provided, shall be punished pursuant to the provisions of Section 3, Act 98 of 1906.

These Regulations shall be in force and effect from and after their adoption and promulgation by the State Board of Health.

The State Board of Health reserves the right conferred on it by Section 2, Act 98 of 1906, "to further revise and amend" whenever the interests of the public health, the advancement of scientific knowledge, or the rulings of the National Food Department make it advisable so to do.

All laws and regulations in conflict with these Regulations are hereby repealed.

Adopted April 25, 1908.



## **MARYLAND.**

### **FRUITS, ETC.**

**SEC. 1. *Fruits and vegetables to be marked.*** All shippers and sellers of all fruits and vegetables in Wicomico county shall be compelled to stamp or mark all baskets, barrels, boxes, packages, crates, parcels or other receptacles used by them for the shipment or sale of any fruit, fruits or vegetables with his, her or their name or names, initials, or with some distinguishing device or mark which may be readily and easily read and seen on the same before such fruit, fruits or vegetables shall be offered for shipment or sale; and if any shipper or seller of any fruit, fruits or vegetables, shall neglect or fail to comply with the provisions of this section, he or she, or they, shall pay a fine of five dollars; said fine to be applied to the public school fund for Wicomico county, but nothing in this Act shall apply to hucksters selling in quantities less than full packages, or to anything delivered to canneries.

**SEC. 2. *Effect.*** This Act shall take effect from May 1, 1908.

Approved April 6, 1908. Laws of 1908, art. 23, ch. 712, p. 1125.

## MASSACHUSETTS.

### GENERAL FOOD LAWS.<sup>a</sup>

SEC. 70. *General inspection authority.* Boards of health of cities and towns, by themselves, their officers or agents, may inspect the carcasses of all slaughtered animals and all meat, fish, vegetables, produce, fruit or provisions of any kind found in their cities or towns, and for such purpose may enter any building, enclosure or other place in which such carcasses or articles are stored, kept or exposed for sale. If, on such inspection, it is found that such carcasses or articles are tainted, diseased, corrupted, decayed, unwholesome or, from any cause, unfit for food, the board of health shall seize the same and cause it or them to be destroyed forthwith or disposed of otherwise than for food. All money received by the board of health for property disposed of as aforesaid shall, after deducting the expenses of said seizure, be paid to the owner of such property. If the board of health seizes or condemns any such carcass or meat for the reason that it is infected with a contagious disease, it shall immediately give notice to the board of cattle commissioners of the name of the owner or person in whose possession it was found, the nature of the disease and the disposition made of said meat or carcass.—*As amended April 17, 1908, Acts and Resolves of 1908, ch. 411, p. 276. See Bul. 69, Rev., pt. 3, p. 266.*

SEC. 72. *Penalty for hindering inspectors.* Whoever prevents, obstructs or interferes with the board of health, its officers or agents, in the performance of its duties as provided herein, or hinders, obstructs or interferes with any inspection or examination by it or them, or whoever secretes or removes any carcass, meat, fish, vegetables, fruit or provisions of any kind, for the purpose of preventing the same from being inspected or examined under the provisions of sections seventy to seventy-six, inclusive, shall be punished by a fine of not more than one hundred dollars or by imprisonment for not more than sixty days, or by both such fine and imprisonment.—*As amended April 17, 1908; Acts and Resolves of 1908, ch. 411, p. 276. See Bul. 69, Rev., pt. 3, p. 266.*

Revised Laws, 1902, vol. 1, ch. 56, p. 555.

SEC. 1. *Repeal.* Sections twenty-five and twenty-six of chapter seventy-five of the Revised Laws (Bul. 69, Rev., pt. 3, p. 248), relating to the sale of adulterated food and drugs, are hereby repealed.

SEC. 2. *Effect.* This act shall take effect upon its passage.

Approved March 18, 1908. Acts and Resolves of 1908, ch. 238, p. 153.

### BREAD.

SEC. 6. *Penalty.* Whoever violates any provision of the preceding three sections shall be punished by a fine of not more than ten dollars for each offence. The sealer of weights and measures in the respective cities and towns, or the commissioner of weights and measures of the commonwealth, shall cause the provisions of the said three sections to be enforced.—*As amended March 10, 1908; Acts and Resolves of 1908, ch. 197, p. 114. See Bul. 69, Rev., pt. 3, p. 252.*

Revised Laws 1902, vol. 1, ch. 57, pp. 557-8.

<sup>a</sup> See also Meat, page 39.

MEAT.<sup>a</sup>

**SEC. 1. *Prohibition; penalty.*** The sale, offer or exposure for sale, or delivery for use as food, of the carcass, or any part or product thereof, of any animal which has come to its death in any manner or by any means otherwise than by slaughter or killing while in a healthy condition, or which at the time of its death is unfit by reason of disease, exhaustion, abuse, neglect or otherwise for use as food, or of any calf weighing less than forty pounds when dressed, with head, feet, hide and entrails removed, is hereby declared to be unlawful and prohibited. Whoever sells or offers or exposes for sale or delivers or causes or authorizes to be sold, offered or exposed for sale or delivered for use as food any such carcass or any part or product thereof, shall be punished by fine of not more than two hundred dollars or by imprisonment for not more than six months.

**SEC. 2. *Inspectors of state and municipal boards of health; seizure and destruction of unlawful products.*** The state board of health and its inspectors, and the state inspectors of health and all boards of health of cities and towns and their inspectors, officers, agents and assistants in their respective districts, shall have and exercise the same powers and duties in and for the enforcement of this act as are at any time conferred or imposed by law upon any board of health, inspector, officer, agent or assistant in respect of any other article or substance the sale or use of which for food is unlawful or prohibited; and it shall be their duty to seize any such carcass or part or product thereof as described in section one hereof, and cause the same to be destroyed forthwith or disposed of otherwise than for food; and all moneys received by any board of health for any property so disposed of shall, after deducting the expenses of such seizure and disposal, be paid to the owner of such property if known.

**SEC. 3. *Places to be inspected.*** Such inspectors, officers, agents and assistants shall visit and keep under observation all places within their respective districts at which neat cattle, sheep, swine or other animals intended for slaughter or for sale or use as food are delivered from transportation, and shall have at all times free access to all such places and to all railroad trains or cars or other vehicles in which such animals may be transported, for the purpose of preventing violations of this act and of detecting and punishing the same.

**SEC. 4. *Powers of inspection.*** The state inspectors of health in their respective districts, and the inspectors appointed by the state board of health for duties relative to the sale of food and drugs, shall have the same rights, powers and authority for and in respect of the inspection, seizure and disposition of all carcasses, meats and provisions which are tainted, diseased, corrupted, decayed, unwholesome, or from any cause unfit for food, or the sale of which for food is unlawful, as are conferred by sections seventy and seventy-one of chapter fifty-six and by section one hundred and two of chapter seventy-five of the Revised Laws, or by other laws, upon boards of health of cities and towns or their inspectors in respect of the articles therein specified; with power to prosecute all offences relating thereto.

**SEC. 5. *Inspection of slaughter houses.*** In addition to the supervision now provided for by law, all slaughter houses shall be under the supervision of the state board of health and subject to inspection by the state inspectors of health in their respective districts.

**SEC. 6. *Amendment.*** Section one hundred and five of chapter seventy-five of the Revised Laws, as amended by section two of chapter three hundred and twelve of the acts of the year nineteen hundred and two, and by section two of

<sup>a</sup> See also General Food Law, page 38.

chapter two hundred and twenty of the acts of the year nineteen hundred and three, is hereby further amended by striking out all after the word "old," in the seventh line, so as to read as follows: Sec. 105. *Exemption.* The provisions of the six preceding sections shall not apply to a person not engaged in such business, who, upon his own premises and not in a slaughter house, slaughters his own neat cattle, sheep or swine, but the carcass of any such animals shall be inspected by an inspector at the time of slaughter, unless said animal is less than six months old.

SEC. 7. *Authority of other officers unimpaired.* Nothing in this act shall affect or impair the rights, powers or authority of any board or officer not herein mentioned.

Approved March 31, 1908. Acts and Resolves of 1908, ch. 329, pp. 218-20.

SEC. 71. *Inspection of veal.* The board of health, by themselves, their officers or agents, may inspect all veal found, offered or exposed for sale or kept with the intent to sell in its city or town, and if, in its opinion, said veal is that of a calf less than four weeks old when killed, the board shall seize and destroy or dispose of it as provided in the preceding section, subject, however, to the provisions thereof relative to the disposal of money.—*As amended April 17, 1908; Acts and Resolves of 1908, ch. 411, p. 276. See Bul. 69, Rev., pt. 3, p. 266.*

Revised Laws, 1902, vol. 1, ch. 56, p. 555.

#### MILK.

SEC. 3. *Unclean vessels without name of owner; penalty.* Every licensed milk dealer who sells, or has in his possession with intent to sell, milk not contained in clean vessels bearing his own name, or the name under which his business is conducted, and bearing no other name, shall be punished by a fine of ten dollars for each offence; but the provisions of this section shall not apply to persons using clean vessels bearing the name of another person whose written permission for such use shall have been obtained previously and registered in the office of the milk inspector, in municipalities having such officer, and in other municipalities registered in the office of the city or town clerk.—*As amended April 22, 1908; Acts and Resolves of 1908, ch. 435, p. 292. See Bul. 104, p. 33.*

Approved March 1, 1906. Acts and Resolves 1906, ch. 116, p. 62.

SEC. 2. *Repeal.* Section four (Bul. 104, p. 33) of said chapter one hundred and sixteen is hereby repealed.

Approved April 22, 1908. Acts and Resolves of 1908, ch. 435, p. 292.

SEC. 12. *Expenditures; report.* The bureau may expend not more than eight thousand dollars annually in its work, and it may co-operate with the state board of health and with inspectors of milk, but it shall not interfere with the duties of such board or officers. It shall annually, before the fifteenth day of January, report to the general court in detail the number of agents, assistants, experts and chemists employed by it, with their expenses and disbursements, of all investigations made by it, of all cases prosecuted with the results thereof, and other information advantageous to the dairy industry.—*As amended April 17, 1908; Acts and Resolves of 1908, ch. 416, p. 278. See Bul. 69, Rev., pt. 3, p. 253.*

Revised Laws 1902, vol. 1, ch. 89, pp. 778-9.

**SEC. 56. Standard for milk.** In prosecutions under the provisions of sections fifty-one to sixty-four, inclusive, milk which, upon analysis, is shown to contain less than twelve and fifteen hundredths per cent of milk solids or less than three and thirty-five hundredths per cent of fat, shall not be considered of good standard quality.—*As amended June 13, 1908; Acts and Resolves of 1908, ch. 643, p. 566. Sec Bul. 69, Rev., pt. 3, p. 258.*

Revised Laws 1902, vol. 1, ch. 56, pp. 547-54.

**SEC. 1. Heated milk; fine if not labeled.** Whoever, himself or by his servant or agent, or as the servant or agent of any person, firm or corporation, sells, exchanges or delivers or has in his custody or possession with intent to sell, exchange or deliver any milk which has been subjected to artificial heat greater than one hundred and sixty-seven degrees Fahrenheit, not having the words "heated milk" distinctly marked upon a light ground in plain black uncondensed gothic letters at least one inch in length in a conspicuous place upon every vessel, can or package from or in which such milk is, or is intended to be, sold, exchanged or delivered shall for a first offence be punished by a fine of not less than fifty nor more than two hundred dollars, for a second offence by a fine of not less than one hundred nor more than three hundred dollars, and for a subsequent offence by a fine of fifty dollars and by imprisonment for not less than sixty nor more than ninety days. If such vessel, can or package is of the capacity of not more than two quarts, said words may be placed upon a detachable label or tag attached thereto and said letters may be less than one inch in length, but not smaller than brier gothic capital letters.

**SEC. 2. Exemptions.** Nothing in this act shall be construed as applying to condensed milk or to milk which has been concentrated to one-half its volume or less.

Approved June 1, 1908. Acts and Resolves of Massachusetts, 1908, ch. 570, p. 404.

#### WATER.

**SEC. 1. Defiling water supply.** Any police officer or constable of a city or town in which any pond, stream or reservoir used for the purpose of domestic water supply is wholly or partly situated, acting within the limits of his city or town, and any executive officer of a water board, board of water commissioners, public institution or water company, furnishing water for domestic purposes, or agent of such water board, board of water commissioners, public institution or water company, duly authorized in writing therefor by such boards, institution or company, acting upon the premises of such board, institution or company and not more than five rods from the water, for such supply may, without a warrant, arrest any person found in the act of bathing in a pond, stream or reservoir, the water of which is used for the purpose aforesaid, and detain him in some convenient place until a complaint can be made against him therefor.

**SEC. 2. Effect.** This act shall take effect upon its passage.

Approved May 26, 1908. Acts and Resolves of 1908, ch. 539, pp. 377-78.

## MISSISSIPPI.

SEC. 2. *Repeal.* That sections \* \* \* 1766 \* \* \* of the Mississippi Code of 1906 be, and the same are hereby, repealed.—*Repealed February 19, 1908; Laws of 1908, ch. 115, p. 118. See Bul. 69, Rev., pt. 4, p. 326.*

Annotated Code, 1892, ch. 37, p. 430, or Code of 1906, sec. 1766.

## NEW JERSEY.

### GENERAL FOOD LAWS.

**SEC. 3. Adulteration defined.** For the purposes of this act an article shall be deemed to be adulterated \* \* \*

In the case of confectionery:

It it contains terra alba, barytes, talc, chrome yellow or other mineral substance, or poisonous color or flavor, or other ingredient deleterious or detrimental to health, or any vinous, malt or spirituous liquor or compound or narcotic drug.

In the case of food:

First. If any substance has been mixed or packed with it so as to reduce or lower or injuriously affect its quality or strength.

Second. If any substance has been substituted wholly or in part for the article.

Third. If any valuable constituent of the article has been wholly or in part abstracted.

Fourth. If it be mixed, colored, powdered, coated or stained in a manner whereby damage or inferiority is concealed.

Fifth. If it contain any added poisonous or other added deleterious ingredient which may render such article injurious to health; *provided*, that when in the preparation of food products for shipment they are preserved by any external application applied in such manner that the preservative is necessarily removed mechanically, or by maceration in water, or otherwise, and directions for the removal of said preservative shall be printed on the covering or the package, the provisions of this act shall be construed as applying only when said products are ready for consumption.

Sixth. If it consists in whole or in part of a filthy, decomposed or putrid animal or vegetable substance, or any portion of an animal unfit for food, whether manufactured or not, or if it is the product of a diseased animal, or one that has died otherwise than by slaughter.—*As amended April 16, 1908, Acts of 1908, ch. 308, pp. 629-630. See Bul. 112, pt. 2, pp. 7-8.*

**SEC. 4. Misbranding defined.** The term "misbranded," as used herein, shall apply to all drugs, or articles of food, or articles which enter into the composition of food, the package or label of which shall bear any statement, design or device regarding such article, or the ingredients or substances contained therein, which shall be false or misleading in any particular, and to any food or drug product which is falsely branded as to the State, Territory or country in which it is manufactured or produced.

For the purposes of this act an article shall also be deemed to be misbranded \* \* \*

In the case of food:

First. If it be an imitation of or offered for sale under the distinctive name of another article.

Second. If it be labeled or branded so as to deceive or mislead the purchaser, or purport to be a foreign product when not so, or if the contents of the package as originally put up shall have been removed, in whole or in part, and other con-

tents shall have been placed in such package, or if it fail to bear a statement on the label of the quantity or proportion of any morphine, opium, cocaine, heroin, alpha or beta eucaine, chloroform, cannabis indica, chloral,<sup>a</sup> hydrate, acetanilide, acetphenetidine, or phenacetin or antipyrin, or any derivative or preparation of any such substances contained therein.

Third. If in package form, and the contents are stated in terms of weight or measure, they are not plainly and correctly stated on the outside of the package.

Fourth. If the package containing it, or its label shall bear any statement, design or device regarding the ingredients or substances contained therein, which statement, design or device shall be false or misleading in any particular.—*As amended April 16, 1908; Acts of 1908, ch. 308, pp. 630-632. See Bul. 112, pt. 2, p. 8.*

SEC. 46. *Guarantee for protection of dealer.* No dealer shall be prosecuted under the provisions of this act for distributing or selling, or having in his possession with intent to distribute or sell, any article of food or drug which under any of said provisions shall be deemed to be adulterated or misbranded; *provided*, that said article of food or drug is distributed or sold or had in possession with intent to distribute or sell in the original unbroken package in which it was received by said dealer, and that, in case said article was purchased by said dealer from a wholesaler, jobber, manufacturer, or other person residing in this State, and said dealer can establish a guarantee signed by such wholesaler, jobber, manufacturer or other person from whom he purchased such article, to the effect that the same is not adulterated or misbranded within the meaning of this act, designating it; or in case said article was purchased by said dealer from a wholesaler, jobber, manufacturer or other person residing in the United States of America, but outside of this State, and said dealer can establish a guarantee, signed by such wholesaler, jobber, manufacturer or other person from whom he purchased such article, to the effect that the same is not adulterated or misbranded within the meaning of an act of the Congress of the United States of America, entitled "An act for preventing the manufacture, sale or transportation of adulterated or misbranded, or poisonous or deleterious foods, drugs, medicines and liquors, and for regulating traffic therein, and for other purposes," approved June thirtieth, one thousand nine hundred and six, and the supplements and amendments thereof. Such guaranty, to afford protection, shall contain the name and address of the person making the sale of such article to such dealer, and in such case said person, if he is a resident of this State, shall be amenable to the prosecution, fines and other penalties which would attach in due course to the dealer under the provisions of this act. If the guaranty is signed by a person who resides outside of this State, then the Board of Health of this State shall report the facts in the case to the Secretary of Agriculture of the United States, or the proper officer appointed for the enforcement of the above-mentioned act of Congress; *and provided further*, that no guarantee that any article is not adulterated or misbranded within the meaning of the above-mentioned act of Congress, shall be effective to exempt any dealer from prosecution under this act, unless the provisions of the above-mentioned act of Congress and of this act covering the adulteration and misbranding of such guaranteed article are identical.

The provisions of this act relating to misbranding shall not apply to the distribution or sale or to the possession with intent to distribute or sell by any dealer of such proprietary foods and medicines as were in such dealer's stock in this State on October first, nineteen hundred and eight; *provided*, that the package or other container in which such foods or medicines shall be contained

<sup>a</sup> So in Statutes.



shall be plainly and conspicuously marked with the words and figures "On hand Oct. 1st, 1908."—*As amended April 16, 1908; Acts of 1908, ch. 308, pp. 632-633. See Bul. 112, pt. 2, pp. 12-13.*

Approved May 20, 1907. Acts of 1907, ch. 217, pp. 485-502.

SEC. 5. *Exemptions for exports; preservatives.* No article shall be deemed to be adulterated or misbranded within the meaning of this act when specially prepared for export to any foreign country, if such article shall be prepared and packed according to the directions of the foreign purchaser, and if no substance is used in the preparation or packing of such article which is prohibited by the laws of the foreign country for export to which said article was prepared; *provided*, that if such article shall be sold or offered for sale for use or consumption within the United States of America, then all the provisions of this act, with regard to adulteration and misbranding, shall apply thereto; *and provided further*, that all food products manufactured in this State during the years one thousand nine hundred and seven and one thousand nine hundred and eight, in which preservatives are used, which preservatives are not now specifically prohibited by the Department of Agriculture of the United States, shall be exempt from the provisions of this act; *provided*, the use of such preservatives is stated upon the label or in branding such products, and also the date of their manufacture.—*As amended April 13, 1908; Acts of 1908, ch. 242, pp. 447-478. See Bul. 112, pt. 2, sec. 6 (5), p. 8.*

Approved May 20, 1907. Laws of 1907, ch. 217, p. 488.

#### CONFECTIONERY.

See General Food Laws, page 43.

#### MILK.

SEC. 6. *Standard; modified milk.* No person shall distribute or sell, or have in his possession with intent to distribute or sell, any milk which contains less than twelve per centum of milk solids, or more than eighty-eight per cent. of watery fluids, or less than three per centum of milk fats; *provided, however*, that it shall not be unlawful for any person to distribute or sell, or have in his possession with intent to distribute or sell, in a container having a capacity of not more than twelve fluid ounces, milk especially prepared for infant or invalid feeding by adding thereto pure water, lime water, milk sugar, cereal starches, or other substances which shall not differ in purity, quality or strength from the standard fixed by this act, or by removing therefrom the sugar or any part thereof, if every such container have blown or moulded in it the words "modified milk" in letters which shall not be less than one-quarter inch in height and the several lines of which shall not be less than one-sixteenth of an inch in width; *and, provided also*, that the milk in such container, before modification, shall have been milk of the standard fixed by this act.—*As amended April 14, 1908; Laws of 1908, ch. 260, p. 551. See Bul. 112, pt. 2, p. 15.*

SEC. 8. *Adulterated or unclean milk prohibited.* No person shall distribute or sell or have in his possession with intent to distribute or sell any milk or cream which contains any water, drug, chemical, preservative, coloring matter, condensed milk, or any substance of any kind or character which has been added thereto or mixed therewith; *provided, however*, it shall not be unlawful for any person to distribute or sell or have in his possession with intent to distribute or sell, any milk or cream modified especially for infant or invalid feeding, by adding thereto or mixing therewith pure water, lime water, milk sugar, cereal starches

or other substances, as provided for in section six of this act, if such modified milk shall be in a container having a capacity of not more than twelve fluid ounces, which container shall be marked as provided for in section six of this act. No person shall distribute or sell, or have in his possession with intent to distribute or sell any milk or cream which is the product in whole or in any part of any animal kept in a crowded, uncleanly or unhealthy place or condition, or which is the product in whole or in part of any animal fed on swill, or any substance in a state of rotteness or putrefaction, or on any substance of an unwholesome nature, or on any food or substance which may produce diseased or unwholesome milk. No person shall distribute or sell, or have in his possession with intent to distribute or sell, any milk or cream which is produced in whole or in part from any animal within fifteen days before or five days after parturition.—*As amended April 14, 1908; Laws of 1908, ch. 260, p. 552. See Bul. 112, pt. 2, p. 15.*

Approved May 20, 1907. Acts of 1907, ch. 217, pp. 488-499.

## NEW YORK.

### DAIRY PRODUCTS.

See Appendix, Bulletin 112, Part II, page 148, for law regulating dairy products, approved July 18, 1907, and included in the compilation for the year ending June 30, 1907, for convenience.

### FRUIT.

SEC. 185. *Other than standard apples prohibited.* No person shall buy for resale, sell, or expose or offer for sale as and for evaporated apples any evaporated apples intended to be used for food, or for consumption by any person other than standard evaporated apples.—*As amended May 23, 1908; Laws of 1908, vol. 2, ch. 486, p. 1704. See Bul. 112, pt. 2, p. 21.*

SEC. 186. *Standard evaporated apples defined.* Evaporated apples containing not more than twenty-seven per centum of water or fluids as determined by drying for four hours at the temperature of boiling water shall be considered standard evaporated apples for the purposes of this act.—*As amended May 23, 1908; Laws of 1908, vol. 2, ch. 486, p. 1704. See Bul. 69, Rev., pt. 5, p. 428.*

SEC. 187. *Only New York fruit to be so labeled.* No person or persons shall sell, offer or expose for sale apples, pears or peaches as and for New York state grown apples, pears or peaches if they were not grown or produced within the state of New York; nor shall they brand or label the package or barrel containing such apples, pears or peaches as New York state apples, pears or peaches if they were not grown or produced within the state of New York. Any person or persons packing or repacking or causing apples or pears to be packed or repacked to be sold upon the markets, shall pack or repack or cause them to be packed or repacked in such a manner that each separate package or barrel shall be packed substantially uniform without intent to deceive the purchaser. Any person, persons or corporation buying from a grower apples or pears which are packed in packages or barrels, marked or labeled with the name of the grower who causes such apples or pears to be repacked in the same packages or barrels or who uses the same packages or barrels for the packing of other fruit or apples or pears shall erase from such package or barrel the name of the grower or packer first or originally placed thereon. But the facing of such package or barrel is not prohibited by this act.—*As amended May 23, 1908; Laws of 1908, vol. 2, ch. 486, pp. 1704-1705. See Bul. 112, pt. 2, p. 21.*

SEC. 188. *"Barrel" defined.* The term "barrel" when used in transactions of purchase or sale of apples, pears or quinces shall represent a quantity equal to one hundred quarts of grain or dry measure and shall be of the following dimensions: head diameter, seventeen and one-eighth inches; length of stave, twenty-eight and one-half inches; bulge, not less than sixty-four inches outside measurement. If the barrel shall be made straight, or without a bulge, it shall contain the same number of cubic inches as the barrel above described. Any person or persons making, manufacturing or causing to be made or manufactured barrels for use in the purchase or sale of apples, pears or quinces, or any person or persons packing apples, pears or quinces in barrels for sale or selling apples, pears or quinces in barrels containing a less quantity than the barrel herein specified shall brand said barrels upon each end and upon the outside, conspicuously, in letters one and one-half inches in length with the words, "short barrel."—*As added May 23, 1908; Laws of 1908, vol. 2, ch. 486, p. 1705. See Bul. 69, Rev., pt. 5, p. 428.*

Laws of 1893, ch. 338; Cumming and Gilbert's General Laws and other General Statutes, Supplement 1904, vol. 4, art. 13, p. 45.

## NORTH CAROLINA.

### GENERAL FOOD LAW.

SEC. 6. *Colors and preservatives prohibited; benzoic and sulphurous acids excepted.* For the purpose of this act an article of food shall be deemed adulterated \* \* \*

Sixth. \* \* \*

If it contain any of the following substances, which are hereby declared deleterious and dangerous to health when added to human food, to-wit: Colors which contain antimony, arsenic, barium, lead, cadmium, chromium, copper, mercury, uranium or zinc; or the following colors: gamboge, corallin, picric acid, aniline, or any of the coal-tar dyes; dulcin, glucin or any other artificially or synthetically prepared substitute for sugar except saccharine; paraffine, formaldehyde, beta-naphthol, abrastol, benzoic acid or benzoates, salicylic acid or salicylates, boric acid or borates, sulphurous acid or sulphites, hydrofluoric or any fluorine compounds, sulphuric acid or potassium sulphate or wood alcohol; *Provided*, that catsups and condimental sauces may, when the fact is plainly and legibly stated in the English language on the wrapper and label of the package in which it is retailed, contain not to exceed two-tenths of one per cent. of benzoic acid or its equivalent in sodium benzoate. Fermented liquors may contain not to exceed two-tenths of one per cent. of combined sulphuric acid, and not to exceed eight-thousandths of one per cent. of sulphurous acid.—*As amended February 1, 1908; Public Laws Extra Session 1908, ch. 117, pp. 130-131. See Bul. 69, Rev., pt. 5, p. 437.*

Approved April 13, 1899. Public Laws 1899, ch. 86, p. 216.

## OHIO.

### GENERAL FOOD LAWS.

**SEC. 1. Adulteration and misbranding prohibited.** That no person shall, within this state, manufacture for sale, offer for sale, sell, deliver or have in his possession with intent to sell or deliver any drug or article of food which is adulterated, within the meaning of this act; that no person shall, within this state, offer for sale, sell, deliver or have in his possession with intent to sell or deliver any drug or article of food which is misbranded, within the meaning of this act.—*As amended May 1, 1908; Laws of 1908 (Senate Bill No. 414), p. 257. See Bul. 69, Rev., pt. 6, p. 459.*

**SEC. 3. Adulteration defined.** An article shall be deemed to be adulterated within the meaning of this act:

(a) In the case of drugs: \* \* \*

(b) In the case of food, drink, flavoring extract, confectionery or condiment: (1) If any substance or substances have been mixed with it, so as to lower or depreciate or injuriously affect its quality, strength or purity; (2) if any inferior or cheaper substance or substances have been substituted wholly, or in part, for it; (3) if any valuable or necessary constituent or ingredient has been wholly, or in part, abstracted from it; (4) if it is an imitation of, or is sold under the name of another article; (5) if it consists wholly, or in part, of a diseased, decomposed, putrid, infected, tainted or rotten animal or vegetable substance or article, whether manufactured or not or, in the case of milk, if it is the produce of a diseased animal; (6) if it is colored, coated, polished or powdered, whereby damage or inferiority is concealed, or if by any means it is made to appear better or of greater value than it really is; (7) if it contains any added substance or ingredient which is poisonous or injurious to health; (8) if, when sold under or by a name recognized in the eight decennial revision of the United States pharmacopœia, or the third edition of the National Formulary, it differs from the standard of strength, quality or purity laid down therein; (9) if, when sold under or by a name not recognized in the eighth decennial revision of the United States pharmacopœia, or the third edition of the National Formulary, but is found in some other pharmacopœia, or other standard work on materia medica, it differs materially from the standard of strength, quality or purity laid down in such work; (10) if the strength, quality or purity falls below the professed standard under which it is sold; (11) if it contains any methyl or wood alcohol.—*As amended May 1, 1908; Laws of 1908 (Senate Bill No. 414), pp. 257-8. See Bul. 69, Rev., pt. 6, pp. 459-60.*

**SEC. 3a. Misbranding defined.** An article shall be deemed to be misbranded within the meaning of this act:

(a) In the case of drugs: \* \* \*

(b) In the case of food, drink, flavoring extracts, confectionery or condiment; (1) If the package fails to bear a statement on the label of the quantity or proportion of any morphine, opium, cocaine, heroine, alpha or beta eucaine, chloroform, cannabis indica, chloral hydrate or acetanilide, or any derivative or preparation of any such substances contained therein; (2) if it be labeled or branded so as to deceive or mislead the purchaser, or purport to be a foreign product when not so; (3) if in package form, and the contents are stated in terms of weight or measure, they are not plainly and correctly stated on the

outside of the package; (4) in case of any flavoring extract, for which no standard exists, if the same is not labeled "artificial" or "imitation" and the formula printed in the same manner hereinafter provided for the labeling of "compounds" or "mixtures" and their formulae; (5) if the package containing it or any label thereon shall bear any statement, design or device regarding it or the ingredients or substances contained therein, which shall be false or misleading in any particular; Provided, that the provision of this act shall not apply to mixtures or compounds recognized as ordinary articles or ingredients of articles of food or drink, if each and every package sold or offered for sale be distinctly labeled in words of the English language as mixtures or compounds, with the name and percentage, in terms of 100 per cent., of each ingredient therein. The word "compound" or "mixture" shall be printed in letters and figures not smaller in either height or width than one-half the largest letter upon any label on the package and the formula shall be printed in letters and not smaller in either height or width than one-fourth the largest upon any label on the package and such compound or mixture must not contain any ingredient that is poisonous or injurious to health.—*Added May 1, 1908; Laws of 1908 (Senate Bill No. 414), pp. 258-9. See Bul. 69, Rev., pt. 6, p. 460.*

SEC. 5. *Penalties.* Whoever refuses to comply, upon demand, with the requirements of section 4, and whoever violates any of the provisions of this act, shall be fined not exceeding one hundred nor less than twenty-five dollars, for the first offense, and for each subsequent offense shall be fined not exceeding two hundred dollars nor less than one hundred dollars, or imprisoned in the county jail not exceeding one hundred, nor less than thirty days, or both. And any person found guilty of manufacturing, offering for sale or selling an adulterated article of food or drug under the provisions of this act, shall be adjudged to pay in addition to the penalties hereinbefore provided for, all necessary costs and expenses incurred in inspecting and analyzing such adulterated articles of which said person may have been found guilty of manufacturing, selling or offering for sale.—*As amended May 1, 1908. Laws of 1908 (Senate Bill No. 414), p. 259. See Bul. 69, Rev., pt. 6, p. 460.*

Passed March 20, 1884. 81 O. L., 67; Laning's Revised Statutes and Recodified Laws, 1905, title 5, ch. 8, pp. 1477-78.

SEC. 3a. *Hindering inspector; penalty.* Any person or persons who refuse to allow said commissioner, or any assistant commissioner or any inspector, or any of his agents entrance to any creamery, factory, store, salesroom, drug store, laboratory, booth, vehicle, steam or electric cars, or place which he desires to enter in the discharge of his official duty; or in any manner interfere with said commissioner, or any assistant commissioner, or any inspector, or agent in the discharge of his official duty; or refuse to deliver to him a sample of any article of food, drug, or linseed oil made, sold, offered or exposed for sale by such person or persons, when the same is requested and when the value thereof is tendered, shall be fined not exceeding two hundred nor less than fifty dollars, for the first offense, and for each subsequent offense shall be fined not exceeding three hundred nor less than one hundred dollars, or imprisoned in the county jail not exceeding one hundred, nor less than thirty days, or both.—*Added May 9, 1908; Laws of 1908 (Senate Bill No. 542), p. 386. See Bul. 69, Rev., pt. 6, p. 461.*

Laning's Revised Statutes and Recodified Laws, 1905, vol. 1, title 3, ch. 22, pp. 193-94.

## DAIRY PRODUCTS.

**SEC. 1. Renovated butter must be so marked.** No person, firm or corporation shall manufacture for sale, offer or expose for sale, sell, exchange or deliver, or have in his possession with the intent to sell, exchange or deliver, any butter that is produced by taking original packing stock butter or other butter, or both, melting the same so that the butter fat can be drawn off or extracted, mixing the said butter fat with skimmed milk, or milk or cream, or other milk product, and rechurning or reworking the said mixture; nor shall any person, firm or corporation manufacture for sale, offer or expose for sale, sell, exchange or deliver, or have in his possession for any such purpose any butter which has been subjected to any process by which it is melted, clarified or refined, and made to resemble butter, and is commonly known as boiled, or cold extracted process or renovated butter, and which for the purpose of this act is hereby designated as "renovated" or "process butter," unless the same shall be branded or marked as provided in section two of this act.

**SEC. 2. Style of label prescribed.** Whoever, himself or by his agent, or as the servant or agent of another person shall sell, expose for sale or have in his custody or possession with intent to sell any "renovated" or "process butter," as defined in section one of this act, shall have the words "renovated butter" or "process butter" conspicuously stamped, labeled or marked in one or two lines and in plain Gothic letters, at least three-eighths of an inch square, so that the words cannot be easily defaced, upon two sides of each and every tub, firkin, box or package containing said "renovated" or "process butter," or, if such butter is exposed for sale uncovered or not in a case or package, a placard containing said words in the same form as above described in this section shall be attached to the mass in such a manner as to be easily seen and read by the purchaser. When "renovated" or "process butter" is sold from such package or otherwise at retail, in print, roll or other form, before being delivered to the purchaser, it shall be wrapped in wrappers plainly stamped on the outside thereof with the words "renovated butter," or "process butter" printed or stamped thereon in one or two lines, and in plain Gothic letters at least three-eighths of an inch square, and such wrapper shall contain no other words or printing thereon and said words "renovated butter" or "process butter" so stamped or printed on the said wrapper shall not be in any manner concealed, but shall be in plain view of the purchaser at the time of the purchase.

**SEC. 3. Penalty.** Any one violating any of the provisions of this act shall for a first offense be punished by a fine of not less than fifty nor more than two hundred dollars; for a second offense by a fine of not less than one hundred nor more than three hundred dollars or by imprisonment in the county jail or workhouse for not less than thirty days nor more than sixty days, or both.

**SEC. 4. Effect.** This act shall take effect sixty days after its passage.

Approved April 30, 1908. Laws of 1908 [Senate Bill No. 478], pp. 243-4.

**SEC. 1. Adulterated or watered milk; penalties.** That whoever by himself or by his servant or agent, or as the servant or agent of any other person, sells, exchanges or delivers, or has in his custody or possession with intent to sell or exchange or exposes or offers for sale or exchange adulterated milk, or milk to which water or any foreign substance has been added, or milk from cows fed on wet distillery waste, or starch waste, or from cows kept in a dairy or place which has been declared to be in an unclean or unsanitary condition by certificate of any duly constituted board of health or duly qualified health officer, within the county in which said dairy is located, or from diseased or sick cows, shall for a first offense, be punished by a fine of not less than fifty nor

more than two hundred dollars: for a second offense, by a fine of not less than one hundred dollars nor more than three hundred dollars, or by imprisonment in the jail or workhouse for not less than thirty nor more than sixty days; and for a subsequent offense, by fine of fifty dollars, and by imprisonment in the jail or workhouse for not less than sixty nor more than ninety days.—*As amended April 30, 1908; Laws of 1908 [Senate Bill No. 359], pp. 239-40. See Bul. 69, Rev., pt. 6, p. 472.*

Passed April 10, 1889, 86 O. L., 229; Laning's Revised Statutes and Recodified Laws, 1905, vol. 1, title 5, ch. 8, p. 1482.

SEC. 1. *Refilling of milk containers.* It shall be unlawful to fill or refill, with milk, cream or other milk product, any glass jar or bottle having the name of any person, firm or corporation blown therein, with intent to sell or vend such milk, cream or other milk product, provided, that the provisions of this section shall not extend to the person, firm or corporation whose name is blown in such glass jar or bottle, or a duly authorized agent or employe thereof.

SEC. 2. *Sterilization of milk containers.* It shall be unlawful to fill or refill, with milk, cream or other milk product, any glass jar or bottle with intent to sell or vend such milk, cream or other milk product, unless such glass jar or bottle be first thoroughly cleansed and sterilized.

SEC. 3. *Penalty.* Any person or persons guilty of violating the provisions of the preceding section of this act shall be fined not more than one hundred dollars.

Approved May 9, 1908. Laws of 1908 (House Bill No. 901), p. 454.

#### VINEGAR.

SEC. 1. *Cider or apple vinegar defined.* That no person shall manufacture for sale, offer, or expose for sale; sell or deliver, or have in his possession with intent to sell or deliver, any vinegar not in compliance with the provisions of this act. Any vinegar manufactured for sale, offered for sale, exposed for sale, sold or delivered, or in the possession of any person with intent to sell or deliver, under the name of cider vinegar, or apple vinegar, or any compounding of the word "cider" or "apple" as the name or part of the name of any vinegar, shall be the product made by the alcoholic and subsequent acetous fermentations of the juice of apples, shall contain no foreign substance, drugs or acids, is lævotatory, and shall contain not less than four (4) grams of acetic acid, not less than 1.6 grams of apple solids, of which not more than fifty (50) per cent. are reducing sugars, and not less than twenty-five hundredths (0.25) grams of apple ash in one hundred cubic centimeters (at a temperature of twenty [20] degrees centigrade); and the water-soluble ash from one hundred (100) cubic centimeters (at a temperature of [20] degrees centigrade) of the vinegar shall contain not less than ten (10) milligrams of phosphoric acid ( $P_2O_5$ ), and which shall require not less than thirty (30) cubic centimeters of decinormal acid to neutralize its alkalinity.

(2) *Wine or grape vinegar defined.* Any vinegar manufactured for sale, offered for sale, exposed for sale, sold or delivered or in the possession of any person with intent to sell or deliver, under the name of wine vinegar, or grape vinegar, shall be the product made by the alcoholic and subsequent acetous fermentations of the juice of grapes, and shall contain, in one hundred (100) cubic centimeters (at a temperature of twenty [20] degrees centigrade), not less than four (4) grams of acetic acid, not less than one (1.0) gram of grape solids, and not less than thirteen hundredths (0.13) grams of grape ash.



(3) *Malt vinegar defined.* Any vinegar manufactured for sale, offered for sale, exposed for sale, sold or delivered or in the possession of any person with intent to sell or deliver, under the name of malt vinegar shall be the product made by the alcoholic and subsequent acetous fermentations, without distillation, of an infusion of barley malt or cereals whose starch has been converted by malt, is dextrorotatory, and shall contain in one hundred (100) cubic centimeters (at a temperature of twenty [20] degrees centigrade), not less than four (4) grams of acetic acid, not less than two (2) grams of solids, and not less than two-tenths (0.2) grams of ash; and the water-soluble ash from one hundred (100) cubic centimeters (at a temperature of twenty [20] degrees centigrade), of the vinegar shall contain not less than nine (9) milligrams of phosphoric acid ( $P_2O_5$ ) and which shall require not less than four (4) cubic centimeters of decinormal acid to neutralize its alkalinity.

(4) *Distilled vinegar defined.* Any vinegar manufactured for sale, offered for sale, exposed for sale, sold or delivered or in the possession of any person with intent to sell or deliver, under the name of distilled vinegar, shall be the product made wholly or in part by the acetous fermentation of dilute distilled alcohol, and shall contain in one hundred (100) cubic centimeters (at a temperature of twenty [20] degrees centigrade), not less than four (4) grams of acetic acid, and shall be free from coloring matter, added during, or after distillation, and from coloring other than that imparted to it by distillation.—*As amended February 28, 1908; Laws of 1908 [Amended House Bill No. 931], pp. 28-9. See Bul. 69, Rev., pt. 6, p. 488.*

SEC. 2. *Labeling of fermented and distilled vinegars; other fermented vinegars; requirements.* All vinegar made by fermentation and oxidation without the intervention of distillation shall be branded "fermented vinegar," with the name of the fruit or substance from which the same is made. And all vinegar made wholly or in part from distilled liquor shall be branded "distilled vinegar," and all such distilled vinegar shall be free from coloring matter added during or after distillation and from color other than that imparted to it by distillation. And all fermented vinegar not otherwise provided for in said section 1, and not being distilled vinegar as defined in said section 1, shall contain not less than two (2) per cent. by weight, upon full evaporation (at the temperature of boiling water) of solids, contained in the fruit or grain or substance from which said vinegar is fermented, and said vinegar shall contain not less than two-and-a-half-tenths of one per cent. ash or mineral matter, the same being the product of the material from which said vinegar is manufactured. And all vinegar shall be made wholly from the fruit or grain from which it purports to be or is represented to be made, and shall contain no foreign substance, and shall contain not less than four per cent., by weight of absolute acetic acid.—*As amended February 28, 1908; Laws of 1908 [Amended House Bill No. 931], p. 29.*

Laning's Revised Statutes and Recodified Laws, 1905, vol. 1, title 5, ch. 8, p. 1499.

# OKLAHOMA.

## GENERAL FOOD LAWS.

SEC. 1. *Personnel of food commission.* A pure food, dairy and drug commission for the State of Oklahoma is hereby created, which shall be composed of the president of the State Board of Agriculture, the secretary of the State Board of Agriculture, the treasurer of the State Board of Agriculture, the State Commissioner of Health and the secretary of the State Board of Pharmacy.

SEC. 2. *Officers of the commission.* The president of said commission shall be the president of the State Board of Agriculture; the secretary of said commission shall be the State Commissioner of Health, and the treasurer of said commission shall be the treasurer of the State Board of Agriculture.

SEC. 3. *Powers and duties of commission; report.* It shall be the duty of said commission to carry into effect the provisions of this Act, and all other Acts in force or which may be hereafter enacted relating to foods, drugs and dairy products, and said commission is hereby authorized and empowered to promulgate and enforce such rules and regulations as they may deem proper and necessary to amend, alter and abolish the same from time to time not inconsistent with the provisions of this Act. They shall also have the power to appoint one dairy inspector, one food inspector, and one drug inspector, to prescribe their duties and powers, and to fix their compensation as hereinafter provided. Said commission shall make an annual report to the Governor on or about the first day of November of each year, giving in a concise manner, in said report, a full statement of the work of said commission, and accounting for all receipts and disbursements of the commission. Said commission shall be authorized and empowered to print their rules, regulations and announcements from time to time as they may deem necessary. The annual report of said commission shall be printed, published and distributed the same as reports of other State commissions. Said commission shall have authority to lease, rent and contract for such office or offices as they may deem necessary for the convenient transaction of the business of said commission at the seat of the State government.

SEC. 4. *Duties of officers.* The president of the commission shall preside at all meetings of the commission and perform such other duties as the commission by their rules may prescribe.

The secretary of the commission shall keep a record of all proceedings of the commission and perform such other duties as are prescribed in this Act, or which may be prescribed by said commission. He shall keep an accurate account of the expenses of said commission and file monthly itemized statements of such expenses with the State Auditor. He shall receive all moneys collected by said commission, and promptly pay the same to the treasurer of said commission, taking a duplicate receipt therefor, one of which shall be filed with the State Auditor, and the other retained by said secretary. He shall make, on the first day of each month, a report to the Governor, covering the entire work of said commission for the preceding month and show, among other things, the number of manufactories<sup>a</sup> and other places inspected, and by whom; the number of specimens of food articles analyzed, and a list of cases

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<sup>a</sup> So in Statutes.

in which adulteration was found, the number of complaints entered against persons for the violation of the law relative to the adulteration of articles named in this Act; the number of convictions had and the amount of fines imposed and collected and sentences passed; and it shall be his duty to cause prosecution to be made against parties violating the provisions of this Act. During the adjournment of the commission the secretary shall be authorized and empowered to carry on the work of the commission.

It shall be the duty of the treasurer to receive, receipt for, and safely keep all funds coming into his hands, and to deposit the same with the State Treasurer at least once each month, taking his receipt therefor, and make to the president of the commission on the first day of each month a full statement of the receipts and disbursements of his office for the month next preceding.

The members of said commission shall make and subscribe to the same oath of office as that prescribed in the Constitution of the State of Oklahoma for other officials, and the secretary and treasurer shall each give bond in the sum of five thousand dollars each for the faithful performance of the duties of their respective offices, which bond shall be approved by the Governor and filed with the Secretary of State.

**SEC. 5. Compensation.** The said board of commissioners shall receive their actual expenses while engaged in the performance of their duties in connection with this Act. They are hereby authorized to employ a stenographer or clerk at a salary not to exceed seventy-five dollars per month, also to fix the compensation of the inspectors, not to exceed three dollars per day and actual expenses.

**SEC. 6. State laboratories for analysis of samples.** For the purpose of this Act there is hereby established two State laboratories for the analysis of food, feeding stuffs, drugs and medicines, which shall be under the supervision of said commission. One of said laboratories shall be established and located at the State University, and the director of said laboratory shall be the professor of the department of chemistry in the State University. The other laboratory shall be established at the State Agricultural and Mechanical College at Stillwater, and the director of said laboratory shall be the chemist of the experiment station in the said Agricultural and Mechanical College. To the said laboratory at the State University all samples of drugs and medicines shall be sent for analysis and examination. And to the said laboratory at the said Agricultural and Mechanical College shall be sent for analysis and examination all samples of foods and feeding stuffs, and all samples of dairy products. The said University and the said Agricultural and Mechanical College shall employ such additional chemists and assistants as are necessary properly and expeditiously to examine and analyze such drugs, medicines, food and dairy products as are sent them by the said commission for the purpose of determining whether such articles are adulterated, misbranded and mislabeled within the meaning of this Act, and if it shall appear that any of such specimens are adulterated, mislabeled or misbranded within the meaning of this Act, the secretary of the commission shall at once certify the facts to the county attorney of the county in which such sample was taken, with a copy of the results of the analysis of the examination of such samples, duly authenticated by the analyst or officer making such examination or analysis, under oath of such analyst or such officer; Provided, that said commission may submit to the department of chemistry at the said State University or at the said experiment station of Agricultural and Mechanical College, any sample or samples of any article of food, drugs, medicines or dairy product for analysis, and the directors of such departments shall make and furnish the commission such analysis or analyses.

The said commission, out of the appropriation hereinafter provided, may employ and fix the compensation of other and additional clerical and professional assistants.

SEC. 7. *Inspection and prosecution.* Said pure food commission is hereby given full jurisdiction over the regulation and control of the manufacture and sale of all foods, drugs and medicines and dairy products, and shall be authorized and empowered to make inspections concerning the purity of the same and to bring prosecutions for violations as provided herein in the case of foods, drugs and dairy products, and shall exercise the necessary police authority in the enforcement of this Act for the preservation of the public health.

SEC. 8. *Adulteration and misbranding prohibited.* The manufacture, production, preparation, compounding, packing, selling, offering or keeping for sale within the State of Oklahoma, or the introduction into the State from any other State or Territory, or the District of Columbia, or from any foreign country of any article of food or dairy product which is adulterated, mislabeled or misbranded within the meaning of this Act is hereby prohibited.

Any person, firm, company or corporation who shall import or receive from any other State, Territory, or the District of Columbia, or from any foreign country, or who having so received, shall deliver, for pay or otherwise, or offer to deliver to any other person any article of food or dairy product mislabeled or misbranded within the meaning of this Act, or any person, firm or corporation who shall manufacture or produce, prepare, compound, pack or sell or offer or keep for sale in the State of Oklahoma any such adulterated, mislabeled or misbranded food or dairy product shall be guilty of a misdemeanor: Provided, that no article of food or dairy product shall be deemed adulterated, mislabeled or misbranded within the provisions of this Act, where prepared for export beyond the jurisdiction of the United States and prepared or packed according to the specifications or directions of the foreign purchaser, when no substance is used in the preparation or packing thereof in conflict with the laws of the foreign country to which said article is intended to be shipped.

SEC. 9. *Term "person" defined.* The word person, as used in this Act, shall be construed to impart the singular and the plural, as the case may demand, and shall include corporations, companies, societies and associations. When construing and enforcing the provisions of this Act, the act, omission or failure of any officer, agent or other person acting for or employed by any corporation, company, society or association, within the scope of employment of his office, shall in every case be also deemed to be the act, omission or failure of such corporation, company, society or association, as well as that of the person.

SEC. 10. *"Food" and "dairy products" defined.* The term "food," as used in this Act, shall include all articles of food, drink, liquor, beverage, confectionery or condiment used by man or other animal, whether simple, mixed or compound. The term "dairy product," as used in this Act, shall include milk, cream, butter, cheese, skimmed milk, buttermilk or any modification of the foregoing materials or compounds containing one or more of same and all products derived from milk.

SEC. 11. *Food standards.* The standard of purity of foods shall be that proclaimed by the Secretary of the Department of Agriculture of the United States.

SEC. 28. *Food adulteration defined.* Food shall be deemed to be adulterated within the meaning of this Act in any of the following cases:

First: If any substance has been mixed or packed with the food so as to reduce or lower or injuriously affect its quality, purity, strength or food value.

Second: If any substance has been substituted wholly or in part for the article of food.

**Third:** If any essential or valuable constituent or ingredient of the article of food has been wholly or partly abstracted.

**Fourth:** If it be mixed, colored, powdered, coated or stained in any manner whereby damage or inferiority is concealed.

**Fifth:** If it contain any added poisonous or other added deleterious ingredient in the food.

**Sixth:** If it consists in whole or in part of a filthy, decomposed or putrid animal or vegetable substance, or any portion of an animal or vegetable unfit for food, whether manufactured or not, or if it is the product of a diseased animal, or one that has died otherwise than by slaughter.

**SEC. 29. Misbranding of food defined.** Food shall be deemed mislabeled or misbranded within the meaning of this Act in any of the following cases:

**First:** If it be in imitation of or offered for sale under the distinctive name of another article of food.

**Second:** If it be labeled, or branded, or colored so as to mislead or deceive the purchaser, or if it be falsely labeled in any respect, or if it purport to be a foreign product when not so, or if the contents of the package as originally put up shall have been removed in whole or in part and other contents shall have been placed in such package.

**Third:** If in package form and the contents stated in terms of weight or measure, they are not plainly and correctly stated on the outside of the package.

**Fourth:** If the package containing it or its label shall bear any statement, design or device regarding the ingredients or the substance contained therein, which statement, design or device shall be false or misleading in any particular.

**Fifth:** When the package bears the name of the manufacturer, jobber or seller, or the grade of the product, it must bear the name of the real manufacturer, jobber or seller, and the true grade or class of the product, the same to be expressed in clear, distinct English words in legible type; Provided, that an article of food shall not be deemed misbranded if it be a well known food product of a nature, quality and appearance, and so exposed to public inspection as not to mislead or deceive or tend to mislead or deceive a purchaser, and not misbranded and not of the character included within the definitions of one to four of this section; Provided, that all packages of imitation butter and cheese shall be so labeled.

**SEC. 30. License for selling proprietary preparations.** \* \* \* Before any manufacturer or proprietor of any food, proprietary or secret preparation, or product of any food or article used in the preparation of food, drug or liquor, or medicine, shall sell, expose or offer for sale or exchange within said State, he shall first procure from the said commission a license or permit to sell the same, and shall pay a filing fee, and for each license or permit so filed in any sum not to exceed \$30.00, as required by said commission, said filing fee to be paid annually.

[Secs. 31-34 relate to drugs.]

**SEC. 35. Misbranding defined.** That the term "misbranded," as used herein, shall apply to all articles which enter into the composition of foods and drugs, the package or label of which shall bear any statement, design or device regarding such article or the ingredients or substances contained therein, which shall be false or misleading in any particular.

**SEC. 36. "Package" defined.** The term "package," as used in this Act, shall be construed to include the original unbroken package, phial, bottle, jar, demi-john, carton, bag, case, can, box, barrel, or any receptacle, vessel or container of whatsoever material or nature which may be used by a manufacturer, pro-

ducer, jobber, packer or dealer for enclosing any article of food or any drug or medicine when exposed or offered for sale.

SEC. 37. *Possession evidence of violation of act.* The possession of any adulterated, mislabeled or misbranded article of food, dairy product or drug, or the offering for sale or the sale of any adulterated, mislabeled or misbranded food, dairy product or drug, by any manufacturer, producer, jobber, packer or dealer in food or drugs, or broker or commission merchant, agent, employee or servant of any such manufacturer, producer, jobber, packer or dealer, shall be prima facie evidence of the violation of this Act.

SEC. 38. *Hotel signs for imitation butter and cheese, adulterated milk and lard.* Whenever any hotel, tavern, restaurant, or boarding house shall knowingly serve for the use of their patrons such food as is defined in this Act as compounds, imitations, blends, renovated butter, imitation cheese, adulterated milk or adulterated lard [they] shall keep conspicuously posted or printed in a bill of fare a list of the articles of food so served in plain and legible words, the brands or labels upon the original package or the constituent parts of such food articles.

SEC. 44. *Imitation honey must be so labeled.* It shall be unlawful for any person to sell, offer, or expose for sale or exchange, any honey which has not been wholly made by bees, unless the same is labelled "imitation" and contains nothing that is injurious to health.

SEC. 47. *Protection of meat and game.* Every dealer or peddler in slaughtered fresh meats, fish, fowl or game for human food, at wholesale or retail in the transportation of such food from place to place, to customers, shall protect the same from dust, flies and other vermin, or substances which may injuriously affect it by securely covering it while being so transported.

SEC. 48. *"Sale" defined.* The taking of orders or the making of agreements or contracts by any person, firm or corporation, or by an agent or representative thereof, for the future delivery of any of the articles, products, goods, wares or merchandise embraced within the provisions of this Act, shall be deemed a sale within the meaning of this Act.

SEC. 49. *Penalty for misbranding or defacing label.* Whoever shall falsely brand, mark, stencil or label any article or product required by this Act to be branded, marked, stenciled or labeled, or shall remove, alter or deface, mutilate, obliterate, imitate, or counterfeit any brand, mark, stencil, or label so required, shall be deemed guilty of a misdemeanor, and upon conviction thereof shall be punished by a fine of not less than fifty dollars nor more than five hundred dollars, or by imprisonment in the county jail for not less than six months nor more than one year, or by both such fine and imprisonment, for each and every offense.

SEC. 51. *Penalty.* Whoever shall do any of the acts or things prohibited or willfully neglect or refuse to do any of the acts or things enjoined by this Act, or in any way violate any of its provisions, shall be deemed guilty of a misdemeanor, and where no specific penalty is prescribed by this Act, shall be punished by a fine of not less than twenty-five nor more than five hundred dollars or by imprisonment in the county jail for a period of not less than thirty days nor more than ninety days, or by both such fine and imprisonment.

SEC. 55. *Colored distilled vinegar illegal.* It shall be unlawful for any person, firm, or corporation to sell or offer for sale in this State, any colored, distilled vinegar.

SEC. 58. *Possession shows intent to commit offense.* If any person shall have in his possession or control any article or articles of adulterated or misbranded or mislabeled food, drugs, or medicines, contrary to the provisions of this Act, he shall be held to have possession of property with intent to use it as a means of

committing a public offense, and all the provisions of the chapter in the statutes of the State of Oklahoma relating to search warrants and proceedings thereby shall apply.

**SEC. 59. Appropriation.** There is hereby appropriated out of the funds in the state treasury not otherwise appropriated, the sum of five thousand dollars, or so much thereof as may be necessary for the purpose of paying the salaries and expenses of the officers created under this Act, and for the maintenance of the State Laboratories created under this Act, and the necessary expenses incurred in the enforcement of this Act.

**SEC. 60. Duties and powers of food inspectors; sheriffs appointed agents; sampling.** It shall be the duty of the pure food inspectors to make, or cause to be made, by one of the directors of the state laboratories examinations and analyses of foods or drugs on sale in Oklahoma, suspected of being adulterated, mislabeled, misbranded, impure or unwholesome, in contravention of the law. And if upon examination or analysis, it is found that said food or drug is adulterated, mislabeled, misbranded, impure or unwholesome, it shall be the duty of the pure food inspector to make complaint against the manufacturer or vendor thereof in the proper county and to furnish the evidence thereon, and thereof to obtain a conviction of the offense charged. And the sheriffs of the respective counties of the State are hereby appointed and constituted agents for the enforcement of this Act, and the pure food inspector or any sheriff shall have free access at all reasonable hours for the purpose of examining any place where it is suspected that any article of adulterated, mislabeled, misbranded, impure or unwholesome food, medicine or drug exists, and such food inspector or sheriff, upon tendering the market price of such article, if a sale be refused, may take from any person, firm or corporation, samples of any article suspected of being adulterated, mislabeled, misbranded, impure or unwholesome, for the purpose of examination or analysis, and divide the said article into three parts, and each part shall be sealed by the pure food inspector or sheriff seizing the said article, with a seal provided for that purpose. If the package be less than four pounds or in volume less than two quarts, three packages of approximately the same size shall be purchased and the marks and tags upon each package noted as above. One shall be delivered to the party from whom purchased, or the party guaranteeing such merchandise, one sample shall be sent or delivered to one of the directors of the state laboratories for examination and analysis, and the third shall be held by the sheriff of the county in which said article was seized, under his seal, for future reference should the case come to trial.

**SEC. 61. Sheriff's fees and expenditures.** For his services hereunder, the sheriff shall be allowed the same fee for travel allowed by law to sheriffs on service of criminal process, together with such compensation as by the board of county commissioners of his county, may be deemed reasonable, and all amounts expended by him in procuring and transmitting the said samples, which fees and amount expended shall be audited and allowed by said board of county commissioners and paid by said county as other bills of said sheriff.

**SEC. 62. Prosecution.** It shall be the duty of all prosecuting officers of the State to prosecute to completion all suits brought under the provisions of this Act, upon the complaint of any member of the pure food, dairy and drug commission or any other citizen of the State of Oklahoma. It shall be the duty of all city and county health officers to take cognizance of and to report all prosecutions or violations of this Act, which may be brought to their notice or they have cognizance of within their jurisdiction.

**SEC. 63. Disposition of fines.** One half of all fines collected by any court or judge for the violation of the provisions of this Act, shall be paid to the state

treasurer, one half shall be paid into the treasury of the county where such cases are prosecuted.

SEC. 64. *Hindering inspectors a misdemeanor; penalty.* It shall be a misdemeanor for any person, firm or corporation to refuse to sell to the pure food inspector, sheriff or agent of the pure food, dairy and drug commission any sample of food or drug suspected of being adulterated, misbranded, mislabeled, impure or unwholesome, upon the tender of the market price thereof, or to conceal such food, liquor, drug or medicine from such officer, or to withhold from him information where such food or drug is kept or stored. Any such person so refusing to sell, or concealing such food, medicine, or drug or withholding such information from said officer upon conviction, shall be punished by a fine of not less than twenty-five dollars, nor more than one hundred dollars, or by imprisonment in the county jail for not less than thirty days nor more than ninety days.

SEC. 65. *Guarantee for protection of dealer.* No dealer shall be prosecuted under the provisions of this Act, when he can establish a guarantee signed by the wholesaler, jobber, manufacturer, or other party residing in the United States from whom he purchased such article to the effect that the same is not adulterated, mislabeled, or misbranded within the meaning of this Act. Said guarantee to afford protection must contain the name and addresses of the party or parties making the sales of such articles to said dealer, and an itemized statement showing the article purchased, or a general guarantee may be filed with the secretary of the United States department of Agriculture, by the manufacturer, wholesaler, jobber, or other party in the United States and given a serial number, which number shall appear on each and every package of goods, sold under such guarantee with the words "guaranteed under the food and drugs Act, June thirtieth, nineteen hundred six." In case the wholesaler, jobber, manufacturer, or other party making such guarantee to such dealer resides without this State, and it appears from the certificate of the director of the state laboratory that such article or articles were adulterated, mislabeled or misbranded, within the meaning of this Act or the "National Pure Food Act" approved June thirtieth, nineteen hundred six, the attorney general of this State must forthwith notify the attorney general of the United States of such violation.

SEC. 66. *Repeal.* All acts and parts of acts in conflict with this Act are hereby repealed.

SEC. 67. *Goods bought prior to passage of law exempt.* That in any prosecution for any violation of any provisions of this Act, relative to the manufacture, possession or sale of any alleged food product or drug, it shall be a valid defense for the defendant to prove that the articles described in the complaint were in his possession as a part of his stock in trade in this State prior to the time of the passage and approval of this Act.

SEC. 68. *Date of effect.* An emergency is hereby declared by reason whereof it is necessary for the immediate preservation of the public health, peace and safety, that this Act take effect from and after its passage and approval.

Approved May 26th, 1908. Session Laws 1907-1908, ch. 37, pp. 403-426.

#### BREAD.

SEC. 55. \* \* \* *Labeling of bread as to time of baking.* It shall be unlawful for any person in this State to sell, or offer to sell any loaf bread, manufactured outside of the State of Oklahoma without having pasted on

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<sup>a</sup> So in Statutes.



each loaf of such bread, a label having written or printed thereon the date and hour of the day the same was baked, and it shall be unlawful to sell any bread over seventy-two hours after the same was baked, without informing each person purchasing or offering to purchase the same that it is "stale bread."

Session Laws 1907-1908, ch. 37, p. 422.

#### CONFECTIONERY.

**Sec. 57. Adulteration of confectionery; penalty.** Any person manufacturing for sale, or selling or offering to sell or exchange any candies, or confectioneries, adulterated by admixture of terra alba, barytes, talc, or other earthly<sup>a</sup> or mineral substances, or any poisonous colors, flavors or extracts, or other deleterious ingredients detrimental to health, shall upon conviction thereof before a court of competent jurisdiction be punished by a fine of not less than ten nor more than one hundred dollars or by imprisonment in the county jail not less than ten days nor more than thirty days, or by both such fine and imprisonment.

Session Laws 1907-1908, ch. 37, p. 423.

#### DAIRY PRODUCTS.<sup>b</sup>

**Sec. 12. Standards for milks and cream.** The following minimum standards of purity for milk and cream are hereby established: Milk shall contain not less than three per centum of butter fat, and cream contain not less than eighteen per centum of butter fat, and it is hereby made unlawful for any person or persons to sell or offer for sale in this State, except under test, any milk or cream falling below said minimum standard therefor. In no event shall milk or cream be sold or offered for sale when produced within thirty days before or fifteen days after calving.

In testing milk or cream for commercial purposes under the provisions of this Act, the same shall be done in accordance with the rules and regulations therefor prescribed by said commission.

All cream sold in the State of Oklahoma shall be tested for butter fat by the following prescribed method:

The Babcock test shall be employed, using a weighed sample of eighteen grams, weighed on a delicate balance and tested in a nine inch bottle, graduated to at least five-tenths per cent of the column of fat read above a temperature of one hundred thirty degrees Fahrenheit. It is hereby made unlawful for any person to test milk or cream at any milk or cream receiving station or at any place where milk or cream is tested for commercial purposes, without first securing a permit issued by said commission. Said commission is hereby authorized to issue to any person making application therefor, a permit to test milk or cream, if, on examination such person be found competent to test milk or cream; said examination shall be given under the direction of said commission at convenient places therefor throughout the State. All permits so issued shall expire on the thirtieth day of June next succeeding the date of issuance.

It is hereby made the duty of said commission to supply to each inspector, a tester under this Act, at the time of issuing to him a license or permit and copy of all rules and regulations formulated by said commission relating to the dairy industry then in force. It shall be unlawful for any owner or employe of any

<sup>a</sup> So in Statutes.

<sup>b</sup> See also General Food Laws, page 54.

creamery or cheese factory, or for any person, to improperly manipulate or under-read the Babcock test.

It shall be unlawful for any person, agent or employe of any creamery, cheese factory or person to so manipulate the sampling that the sample taken does not fairly represent the uniform mixture of the cream or milk from which it was taken.

If milk sold or offered for sale under the provisions of this Act as pure milk, is shown upon analysis by weight, to contain more than eighty-seven and five one-hundredths per centum of watery fluid, or to contain less than twelve and fifty one-hundredths per centum of milk solids, or less than three per centum of butter fat, or if the gravity at sixty degrees Fahrenheit is not between one hundred twenty-nine one-thousandths to one hundred thirty-three one-thousandths, it shall be deemed adulterated. If milks sold or offered for sale under the provisions of this Act as skimmed milk has a gravity at sixty degrees Fahrenheit less than one and thirty-two one-thousandths, and greater than one and thirty-seven one-thousandths, it shall be deemed to be adulterated.

SEC. 13. *Analysis of suspected milks.* Whenever the pure food inspector has reason to believe that any milk found by him is adulterated, he shall take specimens thereof and test the same with such instruments as are used for such purpose, and he shall make an analysis thereof, showing total solids, the percentage of butter, the percentage of water and the percentage of ash, and if the result of such test and analysis indicated that the milk has been adulterated or deprived of its fat below the requirements of section twelve of this Act, the same shall be prima facie evidence of such adulteration in a prosecution under this Act.

SEC. 14. *City milk inspectors.* Authority is hereby given the city council of any city, or the board of trustees of any town or village, to appoint an inspector of milk in any such city or town and to fix his compensation, and when appointed the said inspector of milk shall have all the powers given him by section twenty of this Act, and shall perform all the duties required of inspectors of milk as provided herein, and such other powers and duties as may be conferred or imposed by the ordinances of said cities or towns.

SEC. 15. *Adulteration of milk prohibited; milk defined.* No person shall offer for sale, sell, exchange or deliver or have in his possession with the intent to sell, exchange or deliver, any milk, to which water, chemicals or preservatives, or any other foreign substance has been added. The term "milk," as used in this Act, shall include all milk, cream or milk, in its natural state as drawn from the cow.

SEC. 16. *Adulteration of milk a misdemeanor.* Whoever shall adulterate, by himself, or by his servant or agent, or sell, exchange or deliver, or have in his custody or possession, with intent to sell or exchange the same, or expose or offer for sale, adulterated milk, or milk to which water or any foreign substance or substances in any state of fermentation or putrefication or from any sick or diseased cows shall be guilty of a misdemeanor.

SEC. 17. *Skimmed milk regulations.* Whoever shall adulterate, or cause to be adulterated, sell, exchange or deliver, or have in his custody or possession, with intent to sell or exchange the same or expose or offer for sale or deliver as pure milk, any skimmed milk, from which the cream or any part thereof has been removed, shall be guilty of a misdemeanor.

SEC. 18. *Labeling of skimmed milk.* Any dealer in milk who shall, by himself, servant or agent, sell, exchange or deliver, or have in his custody or possession, with intent to sell, exchange or deliver the same, milk from which the fat has been removed, so as to reduce the same below the requirements of sec-

tion thirteen of this Act, unless in a conspicuous place above the center upon the outside of every vessel, can or package from which any such milk is sold, the words "skimmed milk" are distinctly painted or printed, shall be guilty of a misdemeanor.

SEC. 19. *Manufacturer and dealers in imitation cheese and butter defined; "creamery" and "cheese factory" defined.* Every person who in any manner produces imitation butter or imitation cheese shall be considered a manufacturer thereof.

Any person who sells imitation butter or imitation cheese in packages or quantities containing more than ten pounds, shall be deemed a wholesale dealer thereof.

Any person who deals in imitation butter or imitation cheese in packages containing less than ten pounds each, shall be deemed a retail dealer thereof.

The word "creamery," as used in this Act, is hereby defined as a factory where cream or milk from two or more dairy herds, with or without the addition of salt and coloring matter, is churned into butter. The term "cheese factory," as used in this Act, is hereby defined to be a factory where milk from two or more dairy herds, with or without the addition of salt and coloring matter, is manufactured into cheese. The term "to test milk or cream," as used in this Act, is hereby defined as the process or method by which the percentage of butter fat in said milk or cream is determined.

SEC. 20. *Permits for butter, cheese, and ice cream factories, etc.* It is hereby made unlawful for any manufacturer, wholesale dealer or retail dealer in imitation butter or imitation cheese, or both, to enter upon or engage in the business of producing, manufacturing, handling or selling imitation butter or imitation cheese without first procuring from said commission a permit describing the occupation and place of business of the person engaged in the same, which permit shall expire on the thirtieth day of June following its issuance unless sooner revoked. It is hereby made unlawful to operate any creamery or cheese factory, or both, without first securing from said commission a permit, in which permit shall be described the place of business of the applicant and the business to be conducted under said permit. It is hereby made unlawful for any person to engage in the business of buying cream for any butter, cheese or ice cream factory without first securing from said commission a permit, in which permit shall be described the place of business of the applicant and the business to be conducted under said permit. It is hereby made unlawful for any person engaged in the business of buying cream in this State for the purpose of manufacture or shipment out of the State to make discriminations in the price paid for such cream when purchased upon the same day at different places in said State.

SEC. 21. *Cost of permits.* For permit issued in connection with this Act, there shall be charged and collected annually as follows: From each manufacturer of imitation butter or imitation cheese, the sum of fifty dollars; for each wholesale dealer in imitation butter or imitation cheese, twenty-five dollars; from each retail dealer in imitation butter or imitation cheese, ten dollars; from each creamery or cheese factory, five dollars; from each person engaged in the testing of cream or milk for commercial purposes, one dollar; said fees shall be paid as provided by law, in advance of the issuance of any permit. All permits so issued shall expire on the thirtieth day of June next succeeding the date of issuance. When a permit is issued to such manufacturer, dealer, creamery or factory after the beginning of any license year, the fee charged and collected therefor shall be proportioned to the unexpired portions of such year, counting from the first day of the month in which such license is issued.

SEC. 22. *Unsanitary implements unlawful.* It is hereby made unlawful to use or employ, in and about the keeping or handling of any milk, cream or dairy products to be used as food, any pail, can, vessel, churn, separator or other implement which is in an unclean or unsanitary condition, or to operate any creamery or factory in the manufacture of any dairy products which is in an unclean condition.

SEC. 23. *Diseased cows.* It shall be unlawful knowingly to sell or offer for sale any milk or cream from diseased or unhealthy cows, or from cows kept in a filthy or unsanitary condition, and the pure food, dairy and drug commission is hereby empowered to adopt and promulgate such rules and regulations governing the use of diseased milch cows, and products derived from such cows, and to employ such scientific assistance in the enforcement of said rules and regulations.

SEC. 24. *Duties of chief dairy inspector.* The said chief dairy inspector shall act on all reports and complaints he may receive from the secretary of the commission and from owners or managers of creameries, cheese factories, farmers and others who are interested in dairy products, wherein are reported to him any violations of this Act, or conditions which result in making or rendering dairy products used or to be used for dairy, food or commercial purposes, unclean or unwholesome, and take such action thereon as may be directed by said commission or the secretary thereof and as may be permitted by this Act, or he may deem necessary and proper for improving and advancing the best interests of the dairy industry in this State and public health. He shall also, each month make to the commission a concise report of his transactions as said chief dairy inspector, and make such recommendations in the premises as he shall deem proper and for the better perfection and encouragement of said industry. It shall be the duty of said chief dairy inspector, and such other dairy inspectors as may be appointed by the commission from time to time, to inspect farm dairies, milk and cream receiving stations, creameries, factories and places where dairy products are produced, handled, tested, manufactured, sold or offered for sale and the products thereof, and all utensils, machinery, appliances, implements and methods used or employed in connection therewith.

SEC. 25. *Authority of inspector; sampling.* The said dairy inspector shall have full access, ingress and egress to and from all places where dairy products intended for sale are produced, manufactured, stored, transported, kept or offered for sale. They shall also have the power and authority to open any package, can or vessel containing such products, and may inspect the same and take true samples therefrom for analysis upon paying therefor the full value thereof to the party entitled thereto. Each sample so taken shall be divided into three parts, each equal to the other in amount and quality, two of said parts to be delivered to the chemist of said commission at the Agricultural and Mechanical College at Stillwater, the other sample so taken to be preserved in the office of the commission, and upon application delivered to the person or persons from whom taken when applied for by him, his agent or attorney, provided that said samples shall each be carefully sealed and labeled when and where taken. It shall be unlawful for any person or persons to obstruct, hinder or delay any of said inspectors in the discharge of their official duties.

SEC. 26. *Prosecution.* If it shall appear from the report of the chemist, report of said dairy inspectors, or any of them, that any of the provisions of this Act have been violated, the secretary shall certify the facts to the proper county attorney with a copy of the result of the analysis, if any has been made, duly authenticated to by the chemist under oath. It shall be the duty of every county attorney to whom the secretary shall report any violation of this Act, or any

other Acts relating to dairy products, to cause proceedings to be commenced in the name of the State of Oklahoma and prosecute the same without delay for the recovery of any fines and penalties in such cases provided.

**SEC. 27. "Cream check;" penalty.** It shall be the duty of every person engaged in the buying of cream for manufacture into butter, cheese, ice cream or other products, to give a receipt or "cream check" therefor, clearly and thoroughly stating the name and principal place of business of the person, firm, corporation or association for whom such cream is purchased, and any person, firm, corporation or association who shall violate any of the provisions of this Act shall be deemed guilty of a misdemeanor, and in addition thereto, shall forfeit his license or permit to engage in such business.

**SEC. 56. Each illegal sale of milk a separate offense; hindering inspector.** Each and every quantity of milk sold or exposed for sale or exchange contrary to the provisions of this Act, shall constitute a separate offense.

Any person who shall refuse to permit the pure food inspector, or his assistant, to perform his duty under this Act, either by refusing him entrance to his premises, or by concealing any milk or refusing to permit any animal or milk on premises wherein the animals are kept to be viewed and inspected as herein provided, or by in <sup>a</sup> any manner hindering or resisting any said inspector or assistant inspector in the performance of his duty, shall be guilty of a misdemeanor.

Session Laws 1907-1908, ch. 37, pp. 408-423.

#### FLAVORING EXTRACTS.

**SEC 43. Imitation flavoring extracts must be so labeled.** It shall be unlawful for any person to manufacture, sell or offer for sale or exchange as extracts, flavoring which was not made from the natural fruit unless the same are labelled "Imitation." Provided, that the word "Imitation" must immediately precede the name of the flavoring, in the same type and style. Such flavoring shall be free from coloring matter deleterious to health.

**SEC. 45. Vanilla extract.** It shall be unlawful for any person to manufacture, sell, offer or expose for sale, or exchange, extract of vanilla, essence of vanilla, or spirits of vanilla, not wholly made from the extracted matter of vanilla beans.

Session Laws 1907-1908, ch. 37, p. 420.

#### FLOUR.

**SEC. 52. Labeling of flour compounds.** Within this State no person shall manufacture, offer or expose for sale, keep in his possession with intent to sell or exchange, any flour made from wheat containing any products of corn, rice or other foreign substances, unless each and every package thereof be distinctly and legibly branded or labeled "flour compound" in letters not less than one-half inch in length and be followed with the name of the maker and mill and the location of such flouring mill.

**SEC. 53. Possession of flour compounds evidence of intent to sell.** The having possession of any "flour compound" or "meal compound" which is not branded or labelled as hereinbefore required and directed upon the part of any person engaged in the public or private sale of such article shall, for the purpose of this Act, be deemed prima facie evidence of intent to sell the same.

<sup>a</sup> So in Statutes.

SEC. 54. "*Sale*" defined. The taking of orders or the making of agreements or contracts by any person, firm or corporation, or by an agent or representative thereof, for the future delivery of any "flour compound" or "meal compound" shall be deemed<sup>a</sup> a sale within the meaning of this Act.

Session Laws 1907-1908, ch. 37, p. 422.

#### HONEY.

See General Food Laws, sec. 44, page 58.

#### LARD.

SEC. 39. *Exemptions; lard compound and substitute defined.* The provisions of this Act shall not apply to substances for sale in this state, made in the semblance of lard, if the ingredients or component parts shall consist of pure lard, beef fat or pure stearine and cottonseed oil that is one per cent of the legitimate and exclusive fat of the hog, or pure lard, pure stearine or beef fat, and ninety-nine per cent of cottonseed oil, and the tierce, tub, pail or package containing the same is distinctly and legibly branded, marked or labeled, "lard compound" or "compound lard" or "lard substitute" in letters proportional to the size of the package, and if such mixture contain any other substance than pure lard, pure stearine or beef fat, or pure cottonseed oil, then the person or corporation so manufacturing shall cause the tierce, barrel, tub, pail or package containing the same to be distinctly and legibly branded, marked or labeled "adulterated lard"<sup>a</sup> the term "lard compound" or "compound lard," as used herein, shall include all articles of food used as lard, or made in the semblance of lard, which shall be composed of two or more ingredients or component parts, consisting of either cottonseed oil, pure lard or hog lard, beef fat or pure stearine, the percentage of either of the two or more ingredients used to be in the discretion of the manufacturer. The term "lard substitute," as used herein, shall apply to any compound which may consist of two or more of the aforesaid ingredients or of cottonseed oil alone. Neither shall the provisions of this Act apply to mixtures or compounds consisting of mixtures of beef suet, beef fat or pure stearine, and cottonseed oil, or of cottonseed oil alone, when said mixtures or compounds used as ordinary articles of food, or cooking "compounds" are manufactured and sold under their proper trademark, and when the tierce, barrel, tub, pail or package containing the same shall be distinctly and legibly branded or labeled with the name of the mixture or compound, in letters proportioned to the size of the package, and the name and location of the person, firm or corporation manufacturing the same.

SEC. 40. *Labeling of lard compounds, etc.* Every manufacturer, trader or dealer who, by himself or agent, or as the servant or agent of another person, offers or exposes for sale, or sells or exchanges any form of lard substitute or adulterated lard as hereinbefore defined, shall securely fix or cause to be affixed to the package wherein the name<sup>a</sup> is contained, offered for sale or sold, a label upon the outside and face of which is distinctly and legibly printed in letters not less than one-half inch in length, the words "lard substitute," "adulterated lard" or "lard compound" or other appropriate words which shall correctly express its nature and use.

SEC. 41. *Possession of lard substitute evidence of intent to sell.* The having in possession of any lard substitute or adulterated lard compound, as hereinbefore defined, which is not branded or labeled as hereinbefore required or

<sup>a</sup> So in Statutes.

directed upon the part of any manufacturer, trader or dealer, or any person engaged in the sale of such articles, shall, for the purpose of this Act, be deemed prima facie evidence of intent to sell or exchange the same.

Session Laws 1907-1908, ch. 37, pp. 418-419.

#### MEAT.

See General Food Laws, sec. 47, page 58.

#### PRESERVATIVES.

**SEC. 42. *Preservatives prohibited; exemptions.*** It shall be unlawful for any person to manufacture, sell or expose for sale or exchange any article of food to which has been added formaldehyde, borax, boracic acid, benzoic acid, sulphurous acid, salicylic<sup>a</sup> acid, abradol<sup>a</sup> beta-naphthal,<sup>a</sup> flourine compounds<sup>a</sup> saccharine, alcohol; provided that in the case of molasses and syrups and bleached dried fruits, that in the finished products sulphurous acid, flourine compound<sup>a</sup> and chlorine are entirely removed subject to the rulings of the National Pure Food Commission. Provided, that the spreading of dry borax over the surface of meat cannot be construed to be a violation of this Act.

**SEC. 50. *Preservatives added to oysters, etc.*** Any corporation, firm or person, either in person or by an agent who shall sell or expose for sale within the state of Oklahoma any oysters, clams or other sea food products, to which salicylic<sup>a</sup> acid, formaldehyde or any drug, or other preservative has been added or in preserving which any poisonous or deleterious substance has been used, shall be deemed guilty of a misdemeanor.

Session Laws 1907-1908, ch. 37, pp. 419-421.

#### SEA FOOD.

See Preservatives, sec. 50, above.

#### SPICES AND CONDIMENTS.

**SEC. 46. *Compound spices must be so labeled; terms defined.*** It shall be unlawful for any person to manufacture, sell, offer or expose for sale or exchange to the residents of this state, any spices and condiments,<sup>a</sup> either ground or unground, which are adulterated with any foreign substance or substances within the meaning of this article, which are injurious to health and provided that when foreign substances are used, the package containing said article offered for sale shall contain the word "compound." The term spices and condiments as used herein shall<sup>a</sup> embrace all substances known and recognized in commerce as spices, and used as condiments, whether the same be in natural state or in the form which would result from grinding, milling or mixing, or the compounding of the natural product.

Session Laws 1907-1908, ch. 37, p. 420.

#### VINEGAR.

See General Food Laws, sec. 55, page 58.

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<sup>a</sup> So in Statutes.

## PORTO RICO.

### GENERAL FOOD LAW.

SEC. 1. *Repeal.* Section 336 of the Penal Code is hereby repealed. [See U. S. Dept. Agr., Bureau of Chemistry, Bul. 69, Rev., pt. 7, p. 549.]

Approved March 12, 1908. Laws of 1908, p. 93.

474. *False statements of agents, etc.; false weight or measurement; penalties.* Every commission merchant, broker, agent, factor or consignee, who shall willfully and fraudulently make, or cause to be made, to the principal or consignor of such commission merchant, agent, broker, factor or consignee, a false statement concerning the price obtained for or the quality or quantity of any property consigned or intrusted to such commission merchant, agent, broker, factor or consignee, for sale, shall be deemed guilty of a misdemeanor and on conviction thereof shall be punished by fine not exceeding five hundred dollars, or imprisonment in jail not exceeding six months, or by both such fine and imprisonment. Every person who is putting up in any bale, bag, box, barrel or other package any sugar, tobacco, coffee, rice or other goods usually sold in bales, bags, boxes, barrels or other packages, by weight or otherwise, puts in or conceals therein any extraneous substance whatever for the purposes of fraudulently increasing the weight or measurement of such bale, bag, box, barrel or other package with intent thereby to sell the goods therein, or to enable another to sell the same, for more than the actual weight or measurement of such goods, is punishable by fine not less than twenty-five dollars for such offense, or confined in jail for not less than thirty days, or by both fine and imprisonment in the discretion of the court.—*As amended March 12, 1908; Laws of 1908, p. 93.*

Revised Statutes and Codes of 1902, Penal Code, ch. 8, p. 588.

### MEAT.

SEC. 7. *Use of refrigerated meat in public institutions.* The Director of Health, Charities and Correction, is hereby authorized to determine whether refrigerated meat may or may not be supplied to institutions under his direction.

Approved March 12, 1908. Laws of 1908, p. 74.



## RHODE ISLAND.

### GENERAL FOOD LAWS.

**SEC. 1. *Penalty for adulterating or misbranding; exemption of articles for export.*** It shall be unlawful for any person, firm, or corporation to manufacture, sell, or offer for sale within this state, any drug or article of food which is adulterated or misbranded within the meaning of this act, and any person, firm, or corporation violating any of the provisions of this act shall be guilty of a misdemeanor, and shall, upon conviction, be punished for the first offense by a fine not exceeding fifty dollars, for the second offense by a fine not exceeding one hundred dollars, and for the third and each subsequent offense by a fine of two hundred dollars or imprisonment for one year: *Provided*, that no article shall be deemed misbranded or adulterated within the provisions of this act when intended for export to any foreign country and prepared or packed according to the specifications or directions of the foreign purchaser, when no substance is used in the preparation or packing thereof in conflict with the laws of the foreign country to which said article is intended to be shipped; but if said article shall be in fact sold or offered for sale for domestic use or consumption, then this proviso shall not exempt said article from the operation of any of the other provisions of this act.

[Secs. 2 and 3 pertain to drugs.]

**SEC. 4. *Adulteration defined.*** Food shall be deemed to be adulterated:

**First.**—If any substance has been mixed and packed with it so as to reduce or lower or injuriously affect its quality, strength, or purity. **Second.**—If any substance has been substituted wholly or in part for the article. **Third.**—If any valuable constituent of the article has been wholly or in part abstracted. **Fourth.**—If it is mixed, colored, powdered, coated, stained, or put up in a manner whereby damage or inferiority is concealed. **Fifth.**—If it contains any added poisonous or other added ingredient which may render such article injurious to health: *Provided*, that when in the preparation of food products for shipment they are preserved by any external application applied in such manner that the preservative is necessarily removed mechanically or by maceration in water, or otherwise, and directions for the removal of said preservative shall be printed on the covering of the package, the provisions of this act shall be construed as applying only when said products are ready for consumption. **Sixth.**—If it consists in whole or in part of a filthy, decomposed, or putrid animal or vegetable substance, or any portion of an animal unfit for food, whether manufactured or not, or if it is the product of a diseased animal, or one that has died otherwise than by slaughter.

**SEC. 5. *Adulteration of confectionery defined.*** Confectionery shall be deemed to be adulterated if it contains terra alba, barytes, talc, chrome yellow, or other mineral substances or poisonous colors or flavors, or other ingredients deleterious or detrimental to health, or any vinous, malt, or spirituous liquor or compound or narcotic drug.

SEC. 6. *Misbranding defined.* A drug or an article of food, or an article which enters into the composition of food, shall be deemed to be misbranded :

First.—If the package containing it, or the label on such package, shall bear any statement, design, or device regarding such article, or the ingredients or substances contained therein, which shall be false or misleading in any particular, or if the same is falsely branded as to the state, territory, or country in which it is manufactured or produced. Second.—If it be offered for sale as an imitation of, or under the name of, another article. Third.—If it is in the package form, and the contents are stated in the terms of weight or measure, the same is not plainly and correctly stated on the outside of the package. Fourth.—If the package contains a proprietary or patent medicine, or a proprietary or patent food, and the label fails to bear a statement of the quantity or the proportion of any alcohol, morphine, opium, cocaine, heroin, alpha or beta eucaine, chloroform, cannabis indica, chloral hydrate, or acetanilid or any derivative or preparation of any such substances contained therein: *Provided*, that the provisions of this section shall not apply to the sale and distribution of such proprietary or patent medicines or proprietary or patent foods as were in the possession of any dealer within this state at the time of the taking effect of this law.

SEC. 7. *Guaranty for protection of dealer.* No dealer shall be convicted under the provisions of this act, when he can establish a guaranty, signed by the wholesaler, jobber, manufacturer, or other party residing in the United States, from whom he purchases such articles, to the effect that the same is not adulterated or misbranded within the meaning of the food and drugs act of the United States, approved June 30, 1906, or of this act. Said guaranty, to afford protection, shall contain the name and address of the party or parties making the sale of such articles to such dealer, and in such case said party or parties shall be amenable to the prosecutions, fines, and other penalties which would attach, in due course, to the dealer under the provisions of this act.

SEC. 8. *Sampling; penalty for hindering execution of law.* Every person offering for or exposing for sale or delivering to a purchaser any drug or article of food included in the provisions of this act shall furnish to any commissioner, or other officer or agent appointed hereunder, who shall apply to him for the purpose and shall tender to him the value of the same, a sample or samples, of any drug or article of food which is in his possession, sufficient, after division into two equal or nearly equal parts, for the purpose of analysis. The official or agent thus taking said sample or samples shall then and there, in the presence of the person from whom he obtained it, unless said person refuse to witness the operation, divide said sample or samples into two equal or nearly equal parts or specimens, and seal and label the same, said label to state the kind of food or drugs, the date of such taking, and, if obtainable, the name of the person from whom they were taken; also, if obtainable, the name or names of the parties, if there be any, whom said person represents. Said official or agent shall then and there deliver one of said specimens to the person from whom the same were taken. If any such sample or samples so taken shall appear to be adulterated within the meaning of this act, notice in writing of the fact of such adulteration, containing a description of such sample or samples, shall forthwith be given by mail or otherwise, directed to the person from whom the same were obtained, to the address given by him at the time such sample or samples were taken, before any prosecution shall be instituted thereon: *Provided, however*, that if the person from whom such sample or samples are taken shall omit or refuse to give his name or address, such notice shall not be required. Whoever hinders, obstructs, or in any way interferes with any commissioner or other officer or agent appointed hereunder, in the performance of

his duty, shall, upon conviction, be fined a sum not exceeding one hundred dollars.

**SEC. 9. Seizure.** Any article of food or any drug that is adulterated or misbranded within the meaning of this act shall be liable to be proceeded against in the courts of this state within the county where found, and seized for forfeiture by the same process of law under which liquors illegally sold or for sale may be seized for forfeiture; and if such article or drug is condemned as being adulterated or misbranded or of a poisonous or deleterious character within the meaning of this act, it shall be disposed of by destruction or sale, as the court may direct, and the proceeds thereof, if sold, less the legal costs and charges, shall be paid into the treasury of the state: *Provided, however,* that upon the payment of the costs of such proceedings and the execution and delivery of a good and sufficient bond to the effect that such articles or drugs shall not be sold or otherwise disposed of contrary to the provisions of this act, the court may, by order, direct that such articles or drugs be delivered to the owner thereof. Either party may demand trial by jury of any issue of fact in any such case, and all such proceedings shall be at the suit of and in the name of the state.

**SEC. 10. Soaked canned goods.** All canned articles of food which have been prepared from dried products and have been soaked before canning shall be plainly marked by a brand or label having on its face the word "Soaked," in letters of legible type not smaller than eight-point (Brevier) caps.

**SEC. 11. Board of commissioners.** There shall be a board of food and drug commissioners, consisting of three members, who shall hold office for the term of their appointment, and until their successors, respectively, shall be elected and qualified to act.

At the January session of the general assembly in the year A. D. 1908, the governor, with the advice and consent of the senate, shall appoint three persons to be members of said board, one for a term ending January 31, 1910, one for a term ending January 31, 1912, and one for a term ending January 31, 1914.

At the January session of the general assembly in the year A. D. 1910, and in every second year thereafter, the governor, with the advice and consent of the senate, shall appoint a person to be a member of<sup>a</sup> said board, and the person so appointed shall hold his office until the first day of February in the fifth year of his appointment. Any vacancy which may occur in said board when the senate is not in session shall be filled by the governor until the next session thereof, when he shall, with the advice and consent of the senate, appoint some person to fill such vacancy for the remainder of the term.

**SEC. 12. Duties of board; rules and standards; organization.** It shall be the duty of said board to enforce the provisions of this act. They shall adopt such rules, consistent with the provisions of this act, as may be necessary for its enforcement, and shall adopt rules regulating minimum standards of strength, purity, and quality for food and drugs, defining specific adulterations when such standards are not specified or fixed under this act or by the laws of this state, and subject to the provisions of this act, declaring the proper methods of collecting and examining drugs and articles of food; but such rules and standards shall not be more stringent than, nor conflict with, the rules and standards adopted, or which may hereafter be adopted, for the enforcement of the food and drug act of the United States, approved June 30, 1906, or of any food and drug act of the United States hereafter in force, regulating the misbranding or adulteration of food and drug products for interstate commerce: *Provided, however,* that in prosecutions under this act when the strength, quality, or purity of a drug or an article of food is in issue and the standard of strength,

<sup>a</sup> So in Statutes.

quality, or purity of such drug or article of food is fixed by said board, proof that such drug or article of food is below the standard of strength, quality, or purity fixed by said board shall be evidence that such drug or article of food is adulterated within the meaning of this act.

The said commissioners shall have an office in the state house. They shall be allowed such office, traveling, and personal expenses as may be approved by the governor, to be paid, upon the order of the state auditor, out of any money in the treasury not otherwise appropriated.

They shall meet at least once in three months and as much oftener as may be necessary. They shall proceed to organize by the election of a chairman and an executive secretary, who shall be a practical chemist. Said board shall have authority to appoint such other agents as may be necessary to assist in the enforcement of this act. Said executive secretary and agents shall work under the direction of the said board of commissioners and shall perform such duties as the said board shall prescribe for them to perform.

SEC. 13. *Appropriation.* The sum of thirty-five hundred dollars is hereby appropriated annually, commencing January 1, 1909, from the treasury of the state, to be expended by the board of food and drugs commissioners, for the purpose of meeting the expenses incurred in the enforcement of this act, including fifteen hundred dollars the salary of an executive secretary, the cost of collection of samples, purchase of laboratory supplies, and aid in prosecuting offenders against this act.

SEC. 14. The sum of fifteen hundred dollars or as much thereof as may be necessary, including seven hundred and fifty dollars as recompense for the services of an executive secretary, is hereby appropriated out of the treasury of the state for the purpose of meeting the necessary expense of preparation and notification; and the state auditor is hereby directed to draw his order upon the general treasurer for the payment of the same upon the receipt of vouchers approved by the chairman and secretary of said board.

SEC. 15. *Milk, meat, feeding stuffs, and contagious disease laws not repealed.* This act shall not be construed to repeal Chapter 147 of the General Laws, entitled "Of milk," or any acts in amendment thereof or in addition thereto, or Chapter 131 of the General Laws, entitled "Of the inspection of beef and pork," or any acts in amendment thereof or in addition thereto, or an act entitled "An act authorizing the city of Providence to elect an inspector of beef and pork for said city," passed June 29, 1833, or sections 1 and 2 of Chapter 281 of the Public Laws, entitled "An act in amendment of and in addition to Title XIV, Chapter 74, of the Revised Statutes, 'Of regulations for the prevention of infectious and contagious diseases,'" passed March 5, 1858, or Chapter 631 of the Public Laws, entitled "An act regulating the sale of concentrated commercial feeding stuffs," passed at the January session, 1899.

SEC. 16. *Effect.* Sections 11, 12, and 14 of this act shall take effect immediately, and all other parts of this act shall take effect January 1, 1909.

Approved May 26, 1908. Public Laws passed at the January Session, 1908, ch. 1597, pp. 295-303.

## CANNED GOODS.

See General Food Laws, page 71.

## CONFECTIONERY.

See General Food Laws, page 69.

## SOUTH CAROLINA.

### RICE FLOUR.

**SEC. 1. *Presence of chaff, etc., must be stated on label.*** From and after the approval of this Act it shall be unlawful for any person to sell, or expose for sale, rice flour which contains chaff or any other adulteration, without giving notice by label or otherwise the nature and extent of such adulteration.

**SEC. 2. *Adulterated rice flour liable to seizure.*** Any rice flour so adulterated, which is sold or exposed to sale without being labeled or advertised as such, shall be liable to seizure and sale by any Magistrate having jurisdiction, on the prosecution of any person, the proceeds of such sale to be paid into the Treasury of the County in which such rice flour may be seized.

Approved February 17, 1908. Acts of 1908, No. 476, p. 1053.

## VIRGINIA.

### GENERAL FOOD LAWS.

SEC. 1. *Appointment of dairy and food commissioner.* Within thirty days after this act shall take effect, the governor, by and with the consent of the general assembly in joint session, shall appoint a suitable person to be dairy and food commissioner, which office is hereby created within the department of agriculture and immigration, and which commissioner so appointed shall hold his office until January thirty-one, nineteen hundred and twelve, and until his successor is appointed and qualified. At the regular session of the legislature in nineteen hundred and twelve, and every four years thereafter, the governor, by and with the advice and consent of the general assembly in joint session, shall appoint a dairy and food commissioner, who shall hold his office for the term of four years from the thirty-first day of January, in the year of his appointment, and until his successor is appointed and qualified.

SEC. 2. *Governor may remove commissioner.* The governor shall have the power to remove such commissioner any time, in his discretion, but the reasons for such removal shall be laid before the general assembly in joint session at the next regular or special session of the legislature thereafter; and in case of a vacancy in the office of commissioner from any cause, the governor shall appoint his successor to fill the unexpired term.

SEC. 3. *Oath and bond of commissioner.* Before entering upon the duties of his office, the person so appointed shall make, subscribe and file in the office of the secretary of the Commonwealth, the usual oath of office as provided for in the Constitution of this State, and shall enter into bond, payable to the Commonwealth, in the sum of five thousand dollars, with securities approved by the governor, conditioned for the faithful performance of his duties.

SEC. 4. *Salaries and assistants.* Said dairy and food commissioner shall receive an annual salary of two thousand five hundred dollars. There shall be a deputy dairy and food commissioner, who shall be appointed by the commissioner of agriculture and immigration and the dairy and food commissioner, acting jointly, subject to the confirmation of the State board of agriculture and immigration. The salary of the deputy commissioner shall be fifteen hundred dollars per annum. The said commissioners may also appoint by and with the advice of the board of agriculture and immigration such other special assistants as the proper performance of the duties of the office may require, which special assistants shall be paid for the time actually employed, as said commissioners and board may direct. The persons so appointed shall have power to administer oaths in all matters relative to the dairy and food laws, and shall take and subscribe to the constitutional oath of office, and file the same in the office of the secretary of the Commonwealth; and they shall hold office during the pleasure of the commissioners. The assistants shall have the same right of access to the places to be inspected as the said commissioner. The salaries and expenses authorized by this section shall be for the unexpired part of the fiscal year ending nineteen hundred and eight, and each fiscal year thereafter. Said salaries are to be paid monthly. The salaries and actual and necessary expenses of the said commissioner, deputy commissioner and

assistants, in the performance of their official duties, shall be audited by the State board of agriculture and immigration, and paid upon warrants issued by the dairy and food commissioner upon the State auditor. The board of agriculture and immigration shall provide office room and the necessary furniture and fixtures, and the necessary stationery, supplies and printing for the conduct of the business of said dairy and food commissioner, on his application to said board therefor. Said office shall be, and remain in the city of Richmond.

**SEC. 5. Chemical work.** The chemical work incident to the execution of the dairy and pure food laws shall be done in the chemical laboratory of the department of agriculture and immigration.

**SEC. 6. Duties of commissioner; analyses; inspection; penalty.** It shall be the duty of the dairy and food commissioner to carefully inquire into the dairy and food and drink products, and the several articles which are food or drinks, or the necessary constituents of the food or drinks, which are manufactured or sold, or exposed or offered for sale in this State, and he may, in a lawful manner, procure samples of the same, which shall be duly and carefully examined or analyzed by the State chemist, who shall report to the said commissioner the results of such examination or analyses; and it shall be the duty of the said commissioner to make a complaint against the manufacturer or vendor of any such food or drink or dairy products as are adulterated, impure or unwholesome, in contravention of the laws of this State, and furnish all evidence thereof to obtain a conviction of the offense charged. The dairy and food commissioner or his deputy, or any person appointed by him for that purpose, may make complaint and cause proceedings to be commenced against any person for enforcement of the laws relative to adulteration, impure or unwholesome food or drink, and in such cases he shall not be obliged to furnish security for costs, and shall have power, in the performance of his duties, to enter into any creamery, factory, store, salesroom, drug store, or laboratory, or place where he has reason to believe food and drink is made, stored, sold, or offered for sale, and open any cask, tub, jar, bottle or package containing, or supposed to contain, any article of food or drink, and examine or cause to be examined the contents thereof, and take therefrom samples for analysis. The person making such inspection shall take such samples of such article or product in the presence of at least one witness, and he shall, in the presence of said witness, mark or seal such sample, and shall tender at the time of taking to the manufacturer or vendor of such product, or to the person having the custody of the same, the value thereof, and the statement in writing for the taking of such sample. Whenever it is determined by the dairy and food commissioner, his deputy or assistants, that filthy or unsanitary conditions exist or are permitted to exist in the operation of any bakery, confectionery, or ice cream plant, or at any place where any food or drink products are manufactured, stored or deposited, or sold for any purpose whatever, the proprietor or proprietors, owner or owners of such bakery, confectionery or ice cream plant, or any person or persons owning or operating any plant where any food or drink products are manufactured, stored, deposited or sold, shall be first notified and warned by the said commissioner, his deputy or assistants, to place such bakery, confectionery, or ice cream plant, or any place where any food or drink products are manufactured, stored, deposited or sold, in a sanitary condition within a reasonable length of time; and any person or persons owning or operating any bakery, confectionery or ice cream plant, or any place where any food or drink products are manufactured, stored, deposited or sold, failing to obey such notice and warning, shall be guilty of a misdemeanor, and, upon conviction thereof, shall be punished by a fine of not less than twenty-five dollars nor more than three

hundred dollars and costs of prosecution, or imprisonment in the county or city jail not to exceed ninety days, or until such fine or costs are paid, or both fine and imprisonment, at the discretion of the court.

SEC. 7. *Seizures, sampling, and analysis; prosecution.* The dairy and food commissioner, his deputy, or any person by said commissioner duly appointed for that purpose, is authorized at all times to seize and take possession of any and all food and dairy products, substitutes therefor, or imitation thereof kept for sale, exposed for sale, or held in possession or under the control of any person which in the opinion of said commissioner, or his deputy, or such person by him duly appointed, shall be contrary to the provisions of this act or other laws which now exist or which may be hereafter enacted.

First. The person so making such seizure, as aforesaid, shall take from such goods as seized a sample for the purpose of analysis and shall cause the remainder to be boxed and sealed and shall leave the same in the possession of the person from whom they were seized, subject to such disposition as shall hereafter be made thereof according to the provisions of this act.

Second. The person so making such seizure shall forward the sample so taken to the dairy and food commissioner who shall turn over the same to the State chemist and the said chemist shall certify the results of such analysis, which certificate shall be prima facie evidence of the fact or facts therein certified to, in any court where the same may be offered in evidence.

Third. If upon such analysis it shall appear that said food or dairy products are adulterated, substituted, mis-branded, or imitated within the meaning of this act, said commissioner, or his deputy, or any person by him duly authorized may make complaint before any justice of the peace or police justice having jurisdiction in the city, village or magisterial district, where such goods were seized, and thereupon said justice of the peace shall issue his summons to the person from whom said goods were seized, directing him to appear not less than six or more than twelve days from the date of issuing of said summons and show cause why said goods should not be condemned and disposed of. If the said person from whom said goods were seized cannot be found, the said summons shall be served upon the person then in possession of the goods. The said summons shall be served at least six days before the time of appearance mentioned therein. If the person from whom said goods were seized cannot be found, and no one can be found in possession of said goods, and the defendants shall not appear on the return day, then said justice of the peace shall proceed in said cause in the same manner provided by law where a writ of attachment is returned not personally served upon any of the defendants and none of the defendants shall appear upon the return day.

Fourth. Unless cause to the contrary thereof is shown, or if said goods shall be found upon trial to be in violation of any of the provisions of this act or other laws which now exist or which may be hereafter enacted, it shall be the duty of said justice of the peace or police justice to render judgment that said seized property be forfeited to the State of Virginia, and that the said goods be destroyed or sold by the said commissioner for any purpose other than to be used for food. The mode of procedure before said justice shall be the same as near as may be in civil proceedings before justices of the peace. Either party may appeal to the circuit or corporation courts as appeals are taken from the justices' courts, but it shall not be necessary for the Commonwealth to give any appeal bond.

Fifth. The proceeds arising from any such sale shall be paid into the State treasury and credited to the general fund; provided, that if the owner or party claiming the property or goods so declared forfeited can produce and



prove a written guaranty of purity, signed by the wholesaler, jobber, manufacturer, or other party residing within this State from whom said articles were purchased, then the proceeds of the sale of such articles, over and above the costs of seizure, forfeiture and sale, shall be paid over to such owner or claimant to reimburse him, to the extent of such surplus, for his actual loss resulting from such seizure and forfeiture as shown by the invoice.

Sixth. It shall be the duty of the prosecuting attorney when called upon by said commissioner, or by any person by him authorized as aforesaid, to render any legal assistance in his power in proceeding under the provisions of this act, or any subsequent act relative to the adulteration of food, for the sale of impure or unwholesome food or food products.

SEC. 8. *Annual report; quarterly bulletin.* The dairy and food commissioner shall make an annual report to the commissioner of agriculture and immigration to be, by said commissioner of agriculture and immigration transmitted to the governor on or before the first day of January in each year, and which shall be printed and published on or before the first day of January next thereafter, which report shall cover the doings of his office for the preceding fiscal year, which shall show, among other things, the number of manufactories and other places inspected and by whom, the number of specimens<sup>a</sup> of food articles analyzed and the State chemist's report upon each one; the number of complaints entered against persons for the violating of the laws relative to the adulteration of food, the number of convictions had, and the amount of fines imposed therefor, together with such recommendations relative to the statutes in force as his experience may justify. The dairy and food commissioner shall prepare, print and distribute to all papers of the State, and to such persons as may be interested or may apply therefor, a quarterly bulletin in suitable paper covers, containing results of inspections, the results of analyses made by the State chemist, with the popular explanation of the same, and such other information as may come to him in his official capacity relating to the adulteration of food and drink products and of dairy products, so far as he may deem the same of benefit and advantage to the public; also a brief summary of all the work done during the quarter by the commissioner and his assistants in the enforcement of the laws of the State, but not more than ten thousand copies of such quarterly bulletin shall be printed.

SEC. 9. *Penalty for hindering commissioner.* Any person who shall wilfully hinder or obstruct the dairy and food commissioner, or his deputy or other persons or assistants by him duly authorized, in the exercise of the powers conferred upon him by this act, shall be deemed guilty of a misdemeanor and on conviction shall be punished by a fine of not less than ten dollars nor more than one hundred dollars, or by imprisonment in the county or city jail for not less than ten days nor more than ninety days, or both such fine and imprisonment, in the discretion of the court.

SEC. 10. *Appropriation.* For the purpose of carrying out the provisions of this act the sum of seven thousand five hundred dollars is hereby appropriated for the fiscal year ending February twenty-eighth, nineteen hundred and nine, and in like manner for each fiscal year thereafter, there is hereby appropriated the sum of seven thousand five hundred dollars.

Approved March 11, 1908. Acts of 1908, ch. 188, p. 266.

*Repeal.* An act entitled an act to prevent the sale of adulterated and misbranded foods in the State of Virginia, approved February twenty-seventh,

<sup>a</sup> So in Statutes.

nineteen hundred [Bul. 69 Rev., pt. 8, pp. 639-642], be and the same is, hereby repealed and be it further enacted by the general assembly of Virginia :

SEC. 1. *Sampling and analyses; appointments.* For the purpose of protecting the people of the State from imposition by the adulterating<sup>a</sup> and misbranding of food, the dairy and food commissioner shall cause to be procured from time to time, and under the rules and regulations to be prescribed by him, with the approval of the board of agriculture and immigration in accordance with the provisions of this act, samples of food offered for sale in this State, and shall cause the same to be analyzed and examined microscopically or otherwise by the chemists or other experts of the department of agriculture and immigration; and he is hereby authorized to make such publication of the results of the examination, analyses, and so forth, as he may deem proper; and for the proper execution of the provisions of this act, the dairy and food commissioner shall with the approval of the board make such appointments as may be necessary and the board shall fix the compensation of such appointees.

SEC. 2. *Adulteration a misdemeanor; penalty.* No person, firm or corporation, either directly or through any agent, shall manufacture, sell, expose for sale or have in his possession with intent to sell, any article of food, which is adulterated or misbranded within the meaning of this act, and any person who shall violate any of the provisions of this act shall be guilty of a misdemeanor, and for such offense, shall be fined not exceeding two hundred dollars for the first offense, and for each subsequent offense not exceeding three hundred dollars, or be imprisoned not exceeding one year, or both, in the discretion of the court; and such fines less legal costs and charges, shall be paid into the treasury of the State.

SEC. 3. *Results of analysis as evidence; hearings.* The chemists or other experts of the department of agriculture and immigration shall make, by the methods in use at the time by the association of official agricultural chemists of the United States, examinations of specimens of food offered for sale in Virginia, which may be collected from time to time as prescribed by this act in various parts of the State; and if it shall appear from any such examinations that any such specimen is adulterated or misbranded within the meaning of this act, that notice thereof shall be given to the manufacturer, guarantor, or person from whom the sample was obtained. Any person so notified shall be given an opportunity to be heard under such rules and regulations as may be prescribed by the dairy and food commissioner and the commissioner and board of agriculture and immigration, and if it appears that any of the provisions of this act have been violated, the dairy and food commissioner shall certify the facts to the Commonwealth's attorney of the city or county in which the sample was obtained, and furnish the officer with a copy of the results of the analysis or other examinations of such article, duly authenticated by the analyst or other officer making such examination under the oath of such officer. In all prosecutions arising under this act the certificates of the analyst or other officer making the analysis or examination, when duly sworn to by such officer, shall be prima facie evidence of the fact or facts therein certified.

SEC. 4. *Prosecution.* It shall be the duty of every Commonwealth's attorney to whom the dairy and food commissioner shall report any violation of this act to cause the proceedings to be commenced and prosecuted without delay for the fines and penalties in such cases prescribed.

SEC. 5. *"Food" defined.* The term "food" as used in this act shall include all articles used for food, drink, confectionery, or condiment by man or other animals, whether simple, mixed, or compound.

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<sup>a</sup> So in Statutes.

**SEC. 6. Adulteration defined; confectionery; food.** For the purpose of this act an article shall be deemed to be adulterated:

In the case of confectionery:

First. If it contains terra alba, barytes, talc, chrome yellow, or other mineral substance or poisonous color or flavor, or other ingredient deleterious or detrimental to health, or any vinous, malt, or spirituous liquor or compound or narcotic drug.

In case of other food:

First. If any substance has been mixed or packed with it, so as to reduce or lower or injuriously affect its quality or strength.

Second. If any substance has been substituted wholly or in part for the article.

Third. If any valuable constituent of the article has been wholly or in part abstracted.

Fourth. If it be mixed, colored, powdered, coated, polished or stained in a manner whereby damage or inferiority is concealed.

Fifth. If it contains any added poisonous or other added deleterious ingredient which may render such article injurious to health. Provided, that when in the preparation of food products for shipments they are preserved by any external application in such manner that the preservative is necessarily removed mechanically, or by maceration in water, or otherwise, and directions for the removal of said preservative shall be printed on the covering of the package or furnished with the article, the provisions of this act shall be construed as applying only when said products are ready for consumption.

Sixth. If it consists in whole or in part of diseased, filthy, decomposed, or putrid animal or vegetable matter, or any portion of an animal unfit for food whether manufactured or not, or if it is the product of a diseased animal, or one that had died otherwise than by slaughter.

Seventh. If the containing vessel or any part of it be of such composition as will be acted upon, in the ordinary course of use, by the contents thereof in such a way as to produce an injurious, deleterious, or poisonous compound.

**SEC. 7. Misbranding defined.** The term "misbranded" as used herein shall apply to all articles of food, or articles which enter into the composition of food, the package or label of which shall bear any statement, design or device regarding such article, or the ingredients or substance contained therein, which shall be false or misleading in any particular, and to any food product which is falsely branded as to the State, territory, or country in which it is manufactured or produced.

For the purpose of this act an article shall also be deemed misbranded:

First. If it be an imitation of, or offered for sale under the distinctive name of another article.

Second. If it be labeled or banded <sup>a</sup> so as to deceive or mislead the purchaser, or purport to be a foreign product when not so, or if the contents of the package as originally put up shall have been removed in whole or part, and other contents shall have been placed in such package, or if it fail to bear a statement on the label of the quantity or proportion of any morphine, opium, cocaine, heroin, alpha or beta eucaine, chloroform, cannabis indica, chloral hydrate, or acetanilide or any derivative or preparation of any such substance contained therein.

Third. If in package form, and the contents are stated in terms of weight or measure, they are not plainly and correctly stated on the outside of the package.

Fourth. If the package or its label shall bear any statement, design, or device regarding the ingredients or substance contained therein, which statement,

<sup>a</sup> So in Statutes.

design, or device shall be false or misleading in any particular: Provided, that an article of food which does not contain any added poisonous or deleterious ingredients shall not be deemed to be adulterated or misbranded in the following cases:

First. In the case of mixtures or compounds which may be now or from time to time hereafter known as articles of food under their own distinctive names, and not an imitation of, or offered for sale under the distinctive name of, another article of food, if the name be accompanied on the same label or brand with a statement of the place where said article has been manufactured or produced.

Second. In the case of articles labeled, branded, or tagged so as to plainly indicate that they are compounds, imitations or blends, and having the word "compound," "imitation," or "blend" as the case may be, plainly stated on the package in which such article is offered for sale: provided, the labeling is according to the rules prescribed by the dairy and food commissioner with the approval of the commissioner and board of agriculture and immigration:

Provided, that the term "blend" as used herein shall be construed to mean a mixture of like substances, not excluding harmless coloring or flavoring ingredients used for the purpose of coloring and flavoring only: and provided further that nothing in this act shall be construed as requiring or compelling proprietors or manufacturers of proprietary foods which contain no unwholesome added ingredient to disclose their trade formulas, except in so far as the provisions of this act may require to secure freedom from adulteration and misbranding.

SEC. 8. *Sanitary conditions for handling of human food, especially meats.* It shall be unlawful for any person or persons, firm or corporation, to sell, or to have in his possession with intent to sell for human food, meat or meat food products which has been slaughtered, prepared, or kept where the sanitary conditions, are such that the meat or meat food products are rendered unhealthy, unwholesome, or otherwise unfit for human food.

All peace and health officers shall have the power and are required to seize any animal carcass or parts of carcasses which are intended for sale or offered for sale for human food, which have been slaughtered and prepared, handled or kept under unsanitary conditions, and shall deliver the same forthwith to and before the nearest police judge or justice of the peace, together with all information obtained, and said police judge or said justice of the peace shall, upon sworn complaint being filed, issue warrant for the arrest of all persons who have violated the provisions of this section, and proceed to try the case. Any person, persons, firm or corporation found guilty of violating the provisions of this section shall be fined not less than ten nor more than one hundred dollars, and the meat in question shall be destroyed.

SEC. 9. *Guaranty.* No dealer shall be prosecuted under the provisions of this act when he can establish a guaranty signed by a wholesale dealer, manufacturer or other party, residing in Virginia, from whom he purchased such articles, to the effect that the same is not adulterated or misbranded within the meaning of this act, designating it. Provided, however, that if the article in question is in a broken or open package, said guaranty shall not afford immunity from prosecution, unless such dealer shall furnish satisfactory proof that the article has not been changed in quality. The affidavit of such person shall be accepted as such proof, and the person making such affidavit falsely shall be guilty of perjury, and punished accordingly: Said guaranty, to afford protection, shall contain the name and address of the party or parties making the

sale of such articles to such dealer, and in such cases said party or parties shall be amenable to to<sup>a</sup> the prosecutions, fines, and other penalties which would attach in due course, to the dealer under the provisions of this act: provided, that the above guaranty shall not afford protection to any dealer after the first offense in connection with a product from a particular wholesale dealer or manufacturer.

**SEC. 10. Standards.** The dairy and food commissioner with the approval of the commissioner and board of agriculture and immigration shall from time to time, fix and publish standards or limits of variability permissible in any article of food and these standards when so published shall be the standards before all courts: provided, that when standards have been or may be fixed by the secretary of agriculture of the United States, they shall be accepted by the department of agriculture and immigration and published as standards for Virginia, but said standards shall not go into effect until a reasonable time after publication. The dairy and food commissioner, with the approval of the commissioner and board of agriculture and immigration shall have authority to make uniform rules and regulations for carrying out the provisions of this act.

**SEC. 11. Sampling.** Every person who exposes or offers for sale or delivers to a purchaser any food, shall furnish within business hours and upon tender and full payment of the selling price, a sample of such food, to any person duly authorized to secure the same, and who shall apply to such manufacturer or vendor or person delivering such food to a purchaser for such sample in sufficient quantity for the analysis of such article or articles in his possession. Samples may be purchased on the open market and shall be representative samples; the collector shall also note the name of the vendor and agent through whom the sale was actually made, together with date of purchase, and all samples not taken in unbroken and sealed original packages shall be sealed by the collector in the presence of the vendor with a seal provided for the purpose.

When possible, samples shall be unbroken and sealed original packages, or taken out of unbroken and sealed original packages. Three like samples shall be obtained where the article is in the original package, or, if not in the original package, the sample obtained shall be divided into three equal parts and each part shall be labeled with the marks, brands or tags upon the package, carton, container, wrapper or accompanying printed or written matter. One sample shall be delivered to the party from whom purchased, or to the party guaranteeing such merchandise; two samples shall be sent to the dairy and food commissioner, one of which is to be analyzed, as provided in this act and the other shall be held under seal by the dairy and food commissioner.

**SEC. 12. Penalty for hindering enforcement.** Any manufacturer, dealer or person who refuses to comply upon demand with the requirements of this act or who shall impede, obstruct, hinder or otherwise prevent or attempt to prevent any chemist inspector or other person in the performance of his duty in connection with this act, shall be guilty of a misdemeanor, and upon conviction be fined not less than ten dollars nor more than one hundred dollars, or be imprisoned not more than one hundred days, or both, in the discretion of the court; and said fines, less the legal costs, shall be paid into the treasury of the State.

**SEC. 16. "Person" defined.** The word "person" as used in this act shall be construed to import both the plural and the singular, as the case demands, and shall include partnership, corporations, companies, societies and associations. When construing and enforcing the provisions of this act, the act, omission or

<sup>a</sup> So in Statutes.

failure of any officer, agent or other individual acting for or employed by any partnership, corporation, company, society, or association within the scope of his employment or office, shall in every case be, also deemed the act, omission, or failure of such partnership, corporation, company, society, or association, as well as that of the individual.

SEC. 14. *Seizure and condemnation.* Any person, firm, or corporation who shall manufacture, sell or offer for sale any article of food that is adulterated within the meaning of this act, shall be guilty of a misdemeanor, and in addition to being subject to the penalties already provided in this act, the article of food shall be subject to seizure and condemnation, followed by sale or destruction.

Approved March 14, 1908. Acts of 1908, ch. 372, pp. 654-659.

### CONFECTIONERY.

See General Food Laws, sec. 6, page 79.

### DAIRY PRODUCTS.

SEC. 11. *Investigation of creameries, cheese and milk factories, etc.; assistants.* It shall be the duty of the dairy and food commissioner to foster and encourage the dairy industry of the State, and, for that purpose he shall investigate the general conditions of the creameries, cheese factories, condensed milk factories, skimming stations, milk stations and farm dairies in this State, with full power to enter upon any premises for such investigation, with the object in view of improving the quality and creating and maintaining uniformity of the dairy products of the State; and should it become necessary in the judgment of the dairy and food commissioner, he may cause instruction to be given in any creamery, cheese factory, condensed milk factory, skimming station, milk station or farm dairy, or in any locality of this State, and in order to secure the proper feeding and care of cows, or the practical operation of any plant producing dairy products, and in order to procure such a uniform and standard quality of dairy products in this State, he shall furnish a sufficient number of competent assistants, the appointment of whom is provided for in section four of this act, and they shall be duly qualified to act as such assistants.

SEC. 12. *Penalty for furnishing unclean milk to factories.* Whenever it is determined by the dairy and food commissioner, his deputy or assistants, that any person is using, selling or furnishing to any skimming station, creamery, cheese factory, condensed milk factory, milk depot, farm dairy, milk dealer, the retail trade or to any consumer of milk, any impure or unwholesome milk or cream, which impurity or unwholesomeness is caused by the unsanitary or filthy conditions of the premises where cows are kept or by the unsanitary or filthy care or handling of the cows, or from the use of unclean utensils or from unwholesome food, or from any other cause, the person so using, selling or furnishing to any skimming station, creamery, cheese factory, condensed milk factory, milk depot, farm dairy, milk dealer, the retail trade or to any consumer of milk, any such milk or cream, shall first be notified and warned by the said commissioner, his deputy or assistants not to use, sell or furnish such milk or cream to such skimming station, creamery, cheese factory, condensed milk factory, milk depot, farm dairy, milk dealers, the retail trade or to any consumer of milk, and any person failing to obey such notice and warning and continuing to use, sell or furnish to any skimming station, creamery, cheese

factory, condensed milk factory, farm-dairy, milk dealer or to the retail trade such impure or unwholesome milk or cream, shall be guilty of a misdemeanor, and, upon conviction thereof shall be punished by a fine not less than ten dollars nor more than fifty dollars and costs of prosecution or imprisonment in the county or city jail not to exceed ninety days or until such fine and costs are paid or both fine and imprisonment at the discretion of the court.

SEC. 13. *Penalty for unsanitary conditions of creameries, etc.* Whenever it is determined by the dairy and food commissioner, his deputy or assistants, that unsanitary conditions exist, or are permitted to exist, in the operation of any skimming station, creamery, cheese factory, condensed milk factory, milk depot, or farm dairy, the proprietor or proprietors or manager of said skimming station, creamery, cheese factory, condensed milk factory, milk depot, or farm dairy, shall be first notified and warned by the said commissioner, his deputy or assistants, to place such skimming station in a sanitary condition within a reasonable length of time; and any person or persons owning or operating such skimming station, creamery, cheese factory, condensed milk factory, milk depot, or farm dairy, failing to obey such notices and warnings, shall be guilty of a misdemeanor, and upon conviction thereof, shall be punished by a fine of not less than twenty-five dollars nor more than three hundred dollars, and cost of prosecution, or imprisonment in the county jail not to exceed ninety days, or until such fine and costs are paid, or both fine and imprisonment, at the discretion of the court.

SEC. 14. *Registration of creameries, cheese factories, etc.* It shall be the duty of the proprietor or proprietors of every skimming station, creamery, cheese factory, condensed milk station, or milk depot, in the State where milk or cream is received, by purchase or otherwise, from three or more persons, to register with the dairy and food commissioner, on or before April first of each year, upon blanks furnished by said official, the location of such skimming station, creamery, cheese factory, condensed milk factory, or milk depot, and the name of its owner or owners and manager. And it shall be the duty of the proprietor or proprietors of every skimming station, creamery, cheese factory, condensed milk factory or milk depot, in this State, where milk or cream is received, by purchase or otherwise, from three or more persons, to file a report with the dairy and food commissioner, said report to be made on or before April first of each year, upon blanks furnished by said official, and to show the amount of milk or cream received by said skimming station, creamery, cheese factory, condensed milk factory, or milk depot during the year ending December thirty-first preceding; and said report shall show the amount of butter, cheese, or condensed milk, manufactured during the year, together with a list of the names and post-office addresses of the patrons of said skimming station, creamery, cheese factory, condensed milk factory, or milk depot. Every skimming station, creamery, cheese factory, condensed milk factory, or milk depot, so registering and so reporting, shall pay to the office of the State dairy and food commissioner an annual registration fee of five dollars, to be paid at the time of such registration. The money so collected by the dairy and food commissioner shall be paid into the State treasury, and be used to help defray the expenses of the office of the dairy and food commissioner in addition to the annual appropriation therefor.

SEC. 15. [Relates to commercial feeding stuffs.]

SEC. 16. *Annual report of commissioner.* The published annual report of the dairy and food commissioner, which shall be made to the commissioner of agriculture and immigration, shall include a complete accounting of all moneys received and expended by the said commissioner for the period covered by said report.

SEC. 17. *Enforcement of food laws by dairy and food commissioner.* The enforcement of all existing laws to prevent the manufacture and sale of adulterated and misbranded articles of food heretofore placed under the direction of the commissioner and the board of agriculture and immigration, shall hereafter be placed under the dairy and food commissioner, and shall be enforced by him and under his direction; and all books, papers, and matters referring to the enforcement of such laws shall be transferred to the office of the dairy and food commissioner.

SEC. 18. *Effect.* An emergency existing, because of the large and unlawful sale of adulterated and misbranded food products, this act shall take effect from its passage.

Approved March 11, 1908. Acts of 1908, ch. 188, pp. 266-274.

#### MEATS.

See General Food Laws, sec. 8, page 80.

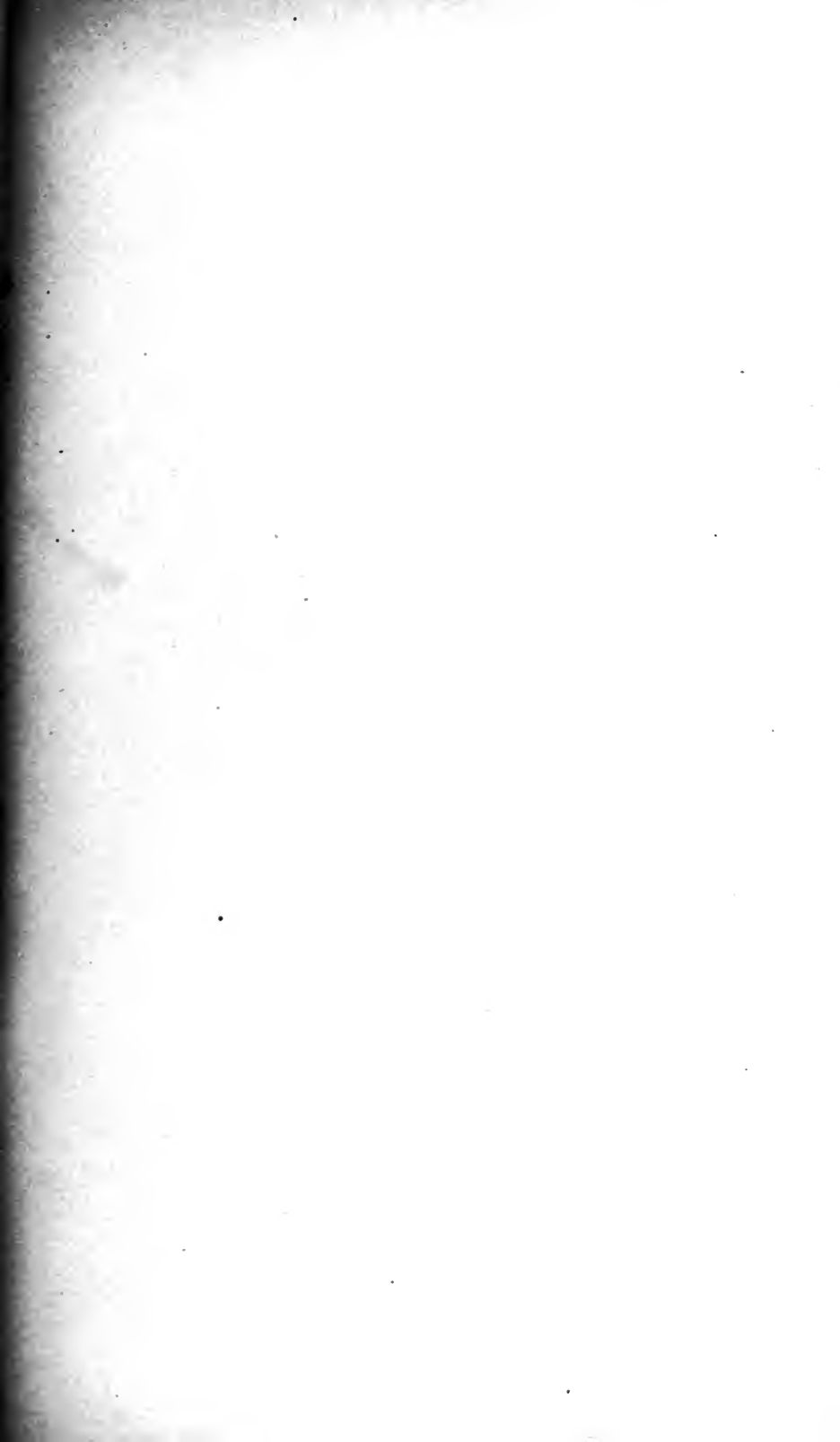


## WISCONSIN.

General food and dairy laws and laws regulating the sale of bread, meat, and sirup, as amended July 9, 1907, are given in Bulletin 112, Part II, page 152, having been included for convenience in that compilation, which covered only laws for the year ending June 30, 1907.









Issued June 7, 1909.

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF CHEMISTRY—BULLETIN No. 122.

H. W. WILEY, Chief of Bureau.

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# PROCEEDINGS

OF THE

TWENTY-FIFTH ANNUAL CONVENTION

OF THE

# ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS,

HELD AT

WASHINGTON, D. C., NOVEMBER 12-16, 1908.

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EDITED BY

HARVEY W. WILEY,

SECRETARY OF THE ASSOCIATION.



GOVERNMENT PRINTING OFFICE.

1909.



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## LETTER OF TRANSMITTAL.

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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF CHEMISTRY,  
*Washington, D. C., January 15, 1909.*

SIR: I have the honor to submit for your approval the Proceedings of the Twenty-fifth Annual Convention of the Association of Official Agricultural Chemists. The reports have been prepared in the most concise form practicable in consideration of the detailed and technical character of the work, all general discussion being practically eliminated. I recommend that these proceedings be published as Bulletin 122 of the Bureau of Chemistry.

Respectfully,

H. W. WILEY,  
*Chief of Bureau.*

HON. JAMES WILSON,  
*Secretary of Agriculture.*



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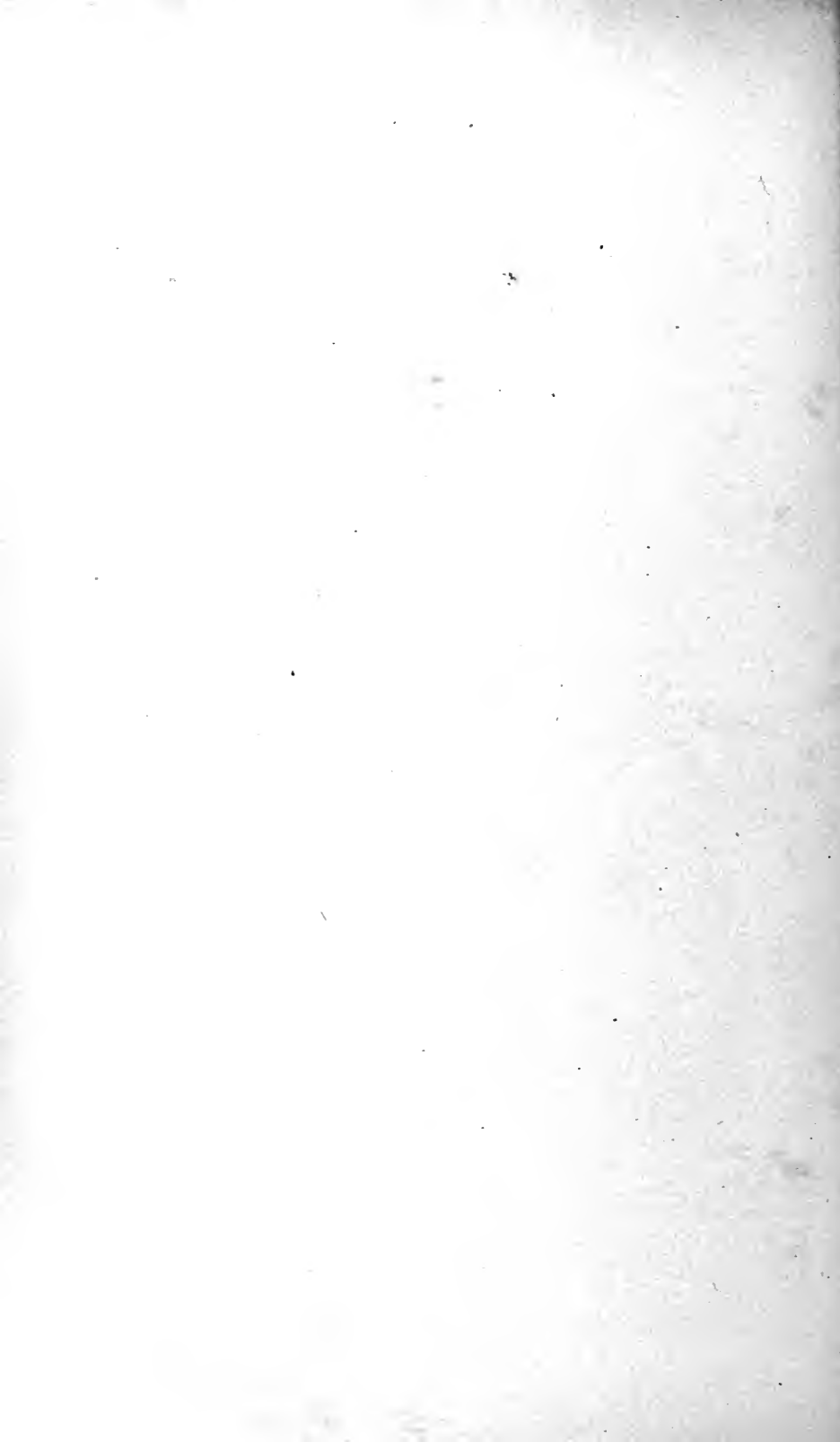
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PROCEEDINGS OF THE TWENTY-FIFTH ANNUAL CONVENTION  
OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

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FIRST DAY.

THURSDAY—MORNING SESSION.

The twenty-fifth annual convention of the Association of Official Agricultural Chemists was called to order by the president, Mr. Harry Snyder, of St. Anthony Park, Minnesota, on the morning of November 12, in the Annex Hall of the Normandie Hotel, Washington, D. C.

Two hundred and sixteen members and visitors registered during the convention, constituting the largest attendance ever recorded. The list is as follows:

MEMBERS AND VISITORS PRESENT.

- Adams, Arthur B., Bureau of Internal Revenue, Washington, D. C.  
Albrech, Maximilian C., U. S. Food and Drug Inspection Laboratory, Pittsburg, Pa.  
Allen, Robert McD., Agricultural Experiment Station, Lexington, Ky.  
Alwood, William Bradford, "Stonehenge" Laboratories, Charlottesville, Va.  
Amoss, Harold L., Bureau of Chemistry, Washington, D. C.  
Averitt, S. D., Agricultural Experiment Station, Lexington, Ky.
- Bailey, Herbert S., Bureau of Chemistry, Washington, D. C.  
Baker, E. L., Geneva, N. Y.  
Balcom, R. Wilfred, Food and Drug Inspection Laboratory, New York, N. Y.  
Barber, Kate G., Bureau of Chemistry, Washington, D. C.  
Barnard, Harry E., State Food and Dairy Commission, Indianapolis, Ind.  
Bartlett, James M., Agricultural Experiment Station, Orono, Me.  
Bates, Carleton, Bureau of Chemistry, Washington, D. C.  
Beal, W. H., Office of Experiment Stations, Washington, D. C.  
Bell, James Munsie, Bureau of Soils, Washington, D. C.  
Bidwell, George L., Bureau of Chemistry, Washington, D. C.  
Bigelow, Willard D., Bureau of Chemistry, Washington, D. C.  
Billings, George A., Department of Agriculture, Washington, D. C.  
Bowker, W. H., Bowker Fertilizer Company, Boston, Mass.  
Boyle, Martin, Bureau of Chemistry, Washington, D. C.  
Boyles, Frank M., Bureau of Chemistry, Washington, D. C.  
Breazeale, James Frank, Bureau of Chemistry, Washington, D. C.  
Breckenridge, John E., American Agricultural Chemical Company, New York, N. Y.

- Bridges, Benjamin H., Food and Drug Analyst to State of Florida, Tallahassee, Fla.  
 Brinton, Clement S., U. S. Food and Drug Inspection Laboratory, Philadelphia, Pa.  
 Broughton, Levin B., Agricultural Experiment Station, College Park, Md.  
 Browne, Charles A., New York Sugar Trade Laboratory, New York, N. Y.  
 Bryan, A. Hugh, Bureau of Chemistry, Washington, D. C.  
 Bryan, Thomas J., State Analyst, Chicago, Ill.  
 Burnet, Wallace C., U. S. Food and Drug Inspection Laboratory, Savannah, Ga.  
 Burnett, Lyle B., Bureau of Chemistry, Washington, D. C.
- Campbell, Walter Gilbert, Bureau of Chemistry, Washington, D. C.  
 Carpenter, Frank B., Virginia-Carolina Chemical Company, Richmond, Va.  
 Carroll, John S., German Kali Works, Atlanta, Ga.  
 Castleman, Philip, Department of Agriculture, Washington, D. C.  
 Cathcart, Charles S., Agricultural Experiment Station, New Brunswick, N. J.  
 Cavanaugh, George W., State College of Agriculture, Cornell University, Ithaca, N. Y.  
 Chace, E. M., Bureau of Chemistry, Washington, D. C.  
 Chapin, Robert M., Bureau of Animal Industry, Washington, D. C.  
 Chesnut, Victor King, Bureau of Chemistry, Washington, D. C.  
 Church, C. G., Bureau of Chemistry, Washington, D. C.  
 Cochran, C. B., Department of Agriculture, West Chester, Pa.  
 Cole, Frank, College Park, Md.  
 Collins, Arthur T., Philadelphia, Pa.  
 Collins, Paul, Agricultural College, College Park, Md.  
 Collins, William Dennis, Bureau of Chemistry, Washington, D. C.  
 Cook, Frank C., Bureau of Chemistry, Washington, D. C.
- Davidson, Robert James, Polytechnic Institute, Washington, D. C.  
 Deemer, Ralph B., College Park, Md.  
 Denis, Willey, Bureau of Chemistry, Washington, D. C.  
 Dietrich, Harry W., Noblesville, Ind.  
 Dodge, C. O., Bureau of Chemistry, Washington, D. C.  
 Donk, M. G., Bureau of Chemistry, Washington, D. C.  
 Doolittle, Roscoe E., U. S. Food and Drug Inspection Laboratory, New York, N. Y.  
 Doran, James M., Bureau of Internal Revenue, Washington, D. C.  
 Dorset, Marion, Bureau of Animal Industry, Washington, D. C.  
 Doyle, Aida M., Bureau of Chemistry, Washington, D. C.  
 Dubois, Wilbur Latimer, U. S. Food and Drug Inspection Laboratory, Buffalo, N. Y.  
 Dunbar, Paul B., Bureau of Chemistry, Washington, D. C.  
 Dunlap, F. L., Bureau of Chemistry, Washington, D. C.
- Eaton, Edgar O., Bureau of Chemistry, Washington, D. C.  
 Edmond, Herman D., Agricultural Experiment Station, Storrs, Conn.  
 Ellett, Walter B., Agricultural Experiment Station, Blacksburg, Va.  
 Emery, James Armitage, Department of Agriculture, Washington, D. C.  
 Emslie, Benjamin Leslie, Toronto, Canada.
- Feldstein, Leonard, Department of Agriculture, Washington, D. C.  
 Fetzer, Lewis William, College Park, Md.  
 Fischer, Louis A., Bureau of Standards, Washington, D. C.  
 Forst, Leo B., Bureau of Internal Revenue, Washington, D. C.  
 Fox, Paul J., Bureau of Chemistry, Washington, D. C.  
 Frear, Julia Reno, State College, Pa.  
 Frear, William, Agricultural Experiment Station, State College, Pa.  
 Fuller, Aubrey V., Bureau of Animal Industry, Washington, D. C.  
 Fuller, F. D., Department of Agriculture, Harrisburg, Pa.  
 Fuller, Henry C., Bureau of Chemistry, Washington, D. C.

- Geisler, Joseph F., State Department of Agriculture, New York, N. Y.  
 Given, Arthur, Bureau of Chemistry, Washington, D. C.  
 Goodnow, Edw. H., Bureau of Chemistry, Washington, D. C.  
 Goodrich, Charles E., Bureau of Chemistry, Washington, D. C.  
 Gore, H. C., Bureau of Chemistry, Washington, D. C.  
 Gudeman, Edward, Chicago, Ill.
- Harcourt, Robert, Ontario Agricultural College, Guelph, Canada.  
 Hart, B. R., U. S. Food and Drug Inspection Laboratory, Cincinnati, Ohio.  
 Hartigan, Will H., Bureau of Chemistry, Washington, D. C.  
 Hartman, Joseph Vance, Washington, D. C.  
 Hartwell, Burt L., Agricultural Experiment Station, Kingston, R. I.  
 Haywood, John K., Bureau of Chemistry, Washington, D. C.  
 Haywood, W. Grimes, Department of Agriculture, Raleigh, N. C.  
 Hillyer, William Eldridge, Bureau of Chemistry, Washington, D. C.  
 Holland, Edw. B., Agricultural Experiment Station, Amherst, Mass.  
 Hite, Bert Holmes, Agricultural Experiment Station, Morgantown, W. Va.  
 Hoover, George W., Bureau of Chemistry, Washington, D. C.  
 Hopkins, Cyril G., Agricultural Experiment Station, Urbana, Ill.  
 Hortvet, Julius, State Dairy and Food Department, St. Paul, Minn.  
 Houghton, Harry W., Bureau of Chemistry, Washington, D. C.  
 Howard, B. J., Bureau of Chemistry, Washington, D. C.  
 Hudson, Claude S., Bureau of Chemistry, Washington, D. C.  
 Humphrey, H. C., Corn Products Refining Company, New York, N. Y.
- Ingersoll, Edwin Henry, Bureau of Animal Industry, Washington, D. C.
- Jacobs, Benjamin Richard, Bureau of Chemistry, Washington, D. C.  
 Jaffa, Myer Edward, State Food and Drug Laboratory, Berkeley, Cal.  
 Jenkins, Lawrence J., Bureau of Chemistry, Washington, D. C.  
 Jones, Charles H., Agricultural Experiment Station, Burlington, Vt.
- Kebler, Lyman F., Bureau of Chemistry, Washington, D. C.  
 Keister, John T., Bureau of Chemistry, Washington, D. C.  
 Kellogg, James W., Pennsylvania Department of Agriculture, Harrisburg, Pa.  
 Kerr, Robert H., Bureau of Animal Industry, Washington, D. C.  
 Knight, George W., Bureau of Chemistry, Washington, D. C.  
 Knight, Henry G., Agricultural Experiment Station, Laramie, Wyo.  
 Knight, Howard L., Office of Experiment Stations, Washington, D. C.
- LaBach, James O., Agricultural Experiment Station, Lexington, Ky.  
 Ladd, Edwin F., Agricultural College, Fargo, N. D.  
 Lane, Clarence B., Dairy Division, Department of Agriculture, Washington, D. C.  
 Leavens, George D., Agricultural Experts' Association, New York, N. Y.  
 Leavitt, Sherman, Bureau of Chemistry, Washington, D. C.  
 Le Clerc, J. A., Bureau of Chemistry, Washington, D. C.  
 Liepsner, F. W., Bureau of Chemistry, Washington, D. C.  
 Linder, William Vanarsdale, Bureau of Internal Revenue, Washington, D. C.  
 Lipman, Jacob G., Agricultural Experiment Station, New Brunswick, N. J.  
 Loomis, Henry M., U. S. Food and Drug Inspection Laboratory, Seattle, Wash.  
 Lowenstein, Arthur, Morris & Company, Chicago, Ill.  
 Lynch, William D., Bureau of Chemistry, Washington, D. C.  
 Lythgoe, Hermann C., State Board of Health, Boston, Mass.

- McCaughey, William John, Bureau of Soils, Washington, D. C.  
 McCormick, R. A., McCormick & Co., Baltimore, Md.  
 McCormick, W. M., McCormick & Co., Baltimore, Md.  
 McDonnell, Henry B., State Chemist, College Park, Md.  
 McGill, A., Inland Revenue, Ottawa, Canada.  
 McKeefe, Edward P., State Department of Agriculture, Albany, N. Y.  
 McNeil, H. C., Bureau of Chemistry, Washington, D. C.  
 Magruder, Egbert Walton, Department of Agriculture, Richmond, Va.  
 Martin, Charles W., Bureau of Chemistry, Washington, D. C.  
 Mason, G. F., H. J. Heinz Company, Pittsburgh, Pa.  
 Michael, Louis G., Agricultural Experiment Station, Ames, Iowa.  
 Miller, Charles C., Philadelphia, Pa.  
 Mitchell, Andrew S., U. S. Food and Drug Inspection Laboratory, St. Paul, Minn.  
 Moore, C. C., Bureau of Chemistry, Washington, D. C.  
 Moore, C. S., Bureau of Chemistry, Washington, D. C.  
 Morgan, Francis Patterson, Bureau of Chemistry, Washington, D. C.  
 Morgan, Will John, Bureau of Chemistry, Washington, D. C.  
 Morse, Fred W., Agricultural Experiment Station, Durham, N. H.  
 Morton, Grant J., Agricultural College, N. D.  
  
 Osborne, N. S., Bureau of Standards, Washington, D. C.  
  
 Palmore, Julian I., Bureau of Chemistry, Washington, D. C.  
 Paradis, Elwood M., Maryland Agricultural College, College Park, Md.  
 Parker, Charles E., Bureau of Chemistry, Washington, D. C.  
 Parkinson, N. A., Bureau of Chemistry, Washington, D. C.  
 Patten, Andrew J., Agricultural Experiment Station, East Lansing, Mich.  
 Payne, Robert L., Baugh & Sons Company, Baltimore, Md.  
 Pennington, Mary E., Food Research Laboratory, Philadelphia, Pa.  
 Pierce, Anne L., Bureau of Chemistry, Washington, D. C.  
 Pinkerton, Thomas C., Baugh & Sons Company, Baltimore, Md.  
 Price, Thomas Malcolm, Bureau of Animal Industry, Washington, D. C.  
 Proctor, M. Fergus, Bureau of Chemistry, Washington, D. C.  
  
 Read, Effie Alberta, Bureau of Chemistry, Washington, D. C.  
 Reed, Edward O., Bureau of Chemistry, Washington, D. C.  
 Richardson, William D., Chicago, Ill.  
 Rieger, John B., Bureau of Chemistry, Washington, D. C.  
 Roark, Ruric Creegan, Bureau of Chemistry, Washington, D. C.  
 Robertson, B. F., Clemson College, S. C.  
 Robb, John Bernard, Department of Agriculture, Richmond, Va.  
 Ross, B. B., State Chemist, Auburn, Ala.  
 Rudnick, Paul, Armour & Co., Chicago, Ill.  
 Rupp, Dr. Philip, Bureau of Chemistry, Washington, D. C.  
 Rusby, Henry H., Newark, N. J.  
  
 Salant, William, Bureau of Chemistry, Washington, D. C.  
 Schorger, Arlie William, Bureau of Internal Revenue, Washington, D. C.  
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 Seil, Harvey A., U. S. Food and Drug Inspection Laboratory, New York, N. Y.  
 Sheib, S. H., Tennessee Chemical Company, Nashville, Tenn.  
 Shepard, James K., Agricultural Experiment Station, Brookings, S. Dak.  
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- Sindall, Harry E., Weikel & Smith Spice Company, Philadelphia, Pa.  
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 Snyder, Harry, Agricultural Experiment Station, St. Anthony Park, Minn.  
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 Stephenson, C. H., Bureau of Chemistry, Washington, D. C.  
 Stewart, Guy Robertson, U. S. Food and Drug Inspection Laboratory, New York, N. Y.  
 Stiles, George W., jr., Bureau of Chemistry, Washington, D. C.  
 Stone, I. Victor, Maryland Agricultural College, College Park, Md.  
 Straughn, M. N., Bureau of Chemistry, Washington, D. C.  
 Street, John Phillips, Agricultural Experiment Station, New Haven, Conn.  
 Sullivan, Arthur L., Bureau of Chemistry, Washington, D. C.
- Taber, Walter C., Bureau of Chemistry, Washington, D. C.  
 Taylor, James Norman, Bureau of Animal Industry, Washington, D. C.  
 Taylor, S. F., 108 Hudson Street, New York, N. Y.  
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 Thatcher, R. W., Agricultural Experiment Station, Pullman, Washington.  
 Tolman, L. M., Bureau of Chemistry, Washington, D. C.  
 Trescot, Thomas Cuthbert, Bureau of Chemistry, Washington, D. C.  
 Trowbridge, P. F., Agricultural Experiment Station, Columbia, Mo.
- Valaer, Peter, jr., Bureau of Internal Revenue, Washington, D. C.  
 Van Slyke, Lucius Lincoln, Agricultural Experiment Station, Geneva, N. Y.  
 Veitch, F. P., Bureau of Chemistry, Washington, D. C.
- Wade, Harold Rollins, Bureau of Chemistry, Washington, D. C.  
 Waggaman, William H., Bureau of Soils, Washington, D. C.  
 Wagner, T. B., Chicago, Ill.  
 Walker, Percy H., Bureau of Chemistry, Washington, D. C.  
 Warren, Lewis E., Bureau of Chemistry, Washington, D. C.  
 Watkins, Howard R., Army Medical Museum, Washington, D. C.  
 Weber, F. C., Bureau of Chemistry, Washington, D. C.  
 Wheeler, H. J., Agricultural Experiment Station, Kingston, R. I.  
 Whitfield, James M., City Board of Health, Richmond, Va.  
 Wiley, H. W., Bureau of Chemistry, Washington, D. C.  
 Wiley, S. W., Wiley & Hoffman, Baltimore, Md.  
 Willard, Julius T., Agricultural Experiment Station, Manhattan, Kans.  
 Wilson, C. P., Bureau of Chemistry, Washington, D. C.  
 Winton, A. L., Food and Drug Inspection Laboratory, Chicago, Ill.  
 Withers, W. A., Agricultural Experiment Station, Raleigh, N. C.
- Young, William J., Bureau of Chemistry, Washington, D. C.

## REPORT ON FOOD ADULTERATION.

By H. F. BARNARD, *Referee*.

The demand for analytical data bearing on the composition of normal and abnormal food products that has arisen in the past three years because of the enactment of food legislation has shown most clearly the absolute necessity for accurate, precise, and at the same time rapid methods for food analysis. It did not at first appear that the

problems of the food analyst were greatly different from those of the analytical chemist, but as the work has developed, a new literature and a new chemistry have by rapid evolution been added to the broad field of chemical science. The boast of the manufacturing chemist that he is always a year in advance of the official chemist who is hunting down iniquities is a constant stimulus and makes necessary the continual development of new methods of analysis and the refinement of old practices.

No radical departure from established methods is advocated by those who have studied the different phases of food adulteration this past year, but the reports of the associate referees show the necessity for continued research.

On fruit products, baking powder and baking chemicals, fats and oils, condiments other than spices, and the determination of water in foods no reports were made. These subjects are all worthy of careful study, and it is to be hoped that this coming year they may be taken up.

I take occasion to call the attention of all food chemists to the imperative necessity for the adoption of uniform methods of analysis which have been proved accurate and reliable. The work of this association is most valuable in providing official methods, but no association can compel chemists to employ standard methods or insist upon more careful analytical work. The food analyst is to-day working constantly in the limelight and his results very frequently are carried to the courts and are subject to the scrutiny of expert chemists and the counsel for the defendant. In too many cases it has appeared that the results of analyses have been published and even used in court which later were found to be inaccurate, thus compelling those responsible for the publication and use of such reports to make public retraction. The value of our work is greatly impaired by the constant recurrence of such mistakes. The necessity for more careful work is well shown by data published in the Proceedings for last year, where chemists analyzing similar condensed milks report an ash content varying from 1.34 to 2.17 per cent and a fat content varying from 7.50 to 9.24 per cent. If the fat in the original milk is determined in these samples on the ash basis, in one case the original milk content is 4.2 per cent fat, in the other case 2.56 per cent, figures which indicate that the same sample of evaporated milk was in one instance made from whole milk and in the other from skimmed milk.

Attention is again called to the fact that we have no satisfactory alcohol table which is accepted by all chemists as a standard. The several alcohol tables now in use, namely, those published in the Official and Provisional Methods of Analysis, the tables given in the United States Pharmacopœia, and those in use by the Internal Revenue Bureau, are not alike. More than that, they are all calculated at 60° F., instead of at the generally accepted standard temperature of 20° C. Can not this association be of assistance to the puzzled chemist who is constantly compelled to recalculate and correct his results and who is confronted in court by alcohol percentages so different from his as to discredit his testimony, but which when calculated to the same basis on the same table are found to be identical?

The Bureau of Standards may well cooperate with the committee from this association for the purpose of revising the alcoholometric tables, and it is recommended that a committee be appointed for this purpose.

## REPORT ON WINE.

By JULIUS HORTVET, *Associate Referee.*

### OUTLINE OF THE WORK.

On February 28, after some preliminary correspondence, the referee on wine sent out the following letter, accompanied by methods of analysis for alcohol, extract, glycerol, ash, fluorids, and total sulphurous acid, substantially as given in Bulletin 107 and in Windisch's *Untersuchung des Weines*. On June 11 these instructions

were supplemented by a further letter submitting a modified method for the determination of volatile and fixed acids. The subjects reported upon included the determination of glycerol in wines, the examination of natural coloring matter in wines, and the determination of total, fixed, and volatile acids; besides which there was submitted a special report on the determination of reducing sugars, by R. M. West. These papers, together with the letter of instructions and a statement of the modified methods which were studied, constitute the report of referee.

## INSTRUCTIONS.

FEBRUARY 28, 1908.

DEAR SIR: I send you herewith an outline of methods for analysis of wine. It is my desire that you subject these methods to careful investigation, using for the purpose samples of your own collection. In general the plan of work for this year will be to allow each collaborator considerable latitude as to how he is to conduct his work. In other words, you are requested to make an independent investigation of all or as many of these methods as possible and prepare a paper giving your results and criticisms. Special attention is directed to the following points:

(1) The change to 20° C. as the standard temperature for specific gravity and alcohol determinations.

(2) Uniformity of terms in which to express results, considering especially (a) the idea of expressing all results when possible in grams per 100 cc of sample, (b) the idea of expressing total, volatile, and fixed acids as cubic centimeters of normal acid in 100 cc of sample.

(3) Improvements in the method for determining glycerol.

(4) A thorough trial of the new method of determining volatile and fixed acids (see below). A drawing and a description of the apparatus used are given in this connection (see page 21).

(5) A criticism of the uranium method for determining phosphoric acid \* \* \*.

(6) A thorough trial of the scheme for examination of the natural coloring matter of wines, using for the purpose samples of the red-wine class.

(7) A criticism of the method for detecting fluorids and the method for determining sulphurous acid.

(8) An investigation and criticism of any of the other provisional methods for wines. Consider especially the volumetric method for determining reducing sugars.

It is desired that the result of your investigations be reported in full, showing all important numerical data, conclusions, and recommendations. \* \* \*

## PROPOSED METHODS FOR THE DETERMINATION OF TOTAL, VOLATILE, AND FIXED ACIDS.

*Total acids.<sup>a</sup>*

Measure 10 cc of the sample into a 300 cc flask, add 100 to 200 cc of recently boiled distilled water, according to the color of the wine, and boil three minutes under a reflux condenser. After cooling add 2 or 3 drops of phenolphthalein and titrate with tenth-normal sodium hydroxid. Express the result for total acids as cubic centimeters of normal acid in 100 cc of the wine.

*Volatile and fixed acids.*

The apparatus to be used consists of a cylindrical flat bottomed flask of about 300 cc capacity, provided with an elongated wide neck. Into the neck of this flask is fitted by means of a short section of thick rubber tubing a cylindrical shaped flask in the bottom of which is a small opening leading inward through a siphon-shaped tube bent back upon itself and terminating at a point close to the bottom. The inner flask is connected to a condenser by means of a bent tube and safety bulb. In the stopper is also fitted a small funnel provided with a glass stop-cock. The distillate from the condenser is received in a cylindrical graduate.

Pour 100 cc of recently boiled distilled water into the larger flask, tightly fit the smaller flask into the wide neck, run in 10 cc of wine through the funnel, rinse out the funnel with a little water, close the stop-cock and heat the water to boiling. The steam passing through the siphon tube and through the wine carries out the volatile acids. When 50 cc of distillate have passed over, empty the graduate and continue the

<sup>a</sup> Pierre Breteau, Guide pratique des falsifications et alterations des substances alimentaires, p. 318.

distillation. Titrate the 50 cc distillate with one-tenth normal sodium hydroxid, using phenolphthalein as an indicator.

Stop the distillation when an additional 10 cc of distillate requires only one drop of the standard alkali solution to neutralize. Usually 80 cc of distillate will include practically all of the volatile acids.

On cooling the apparatus the liquid remaining in the inner flask is siphoned into the outer flask. Rinse out the remaining small amount of sample by running several portions of hot water through the funnel tube and disconnect the two flasks. In case of a light colored wine or a white wine, add 100 to 200 cc of recently boiled distilled water and titrate with one-tenth normal sodium hydroxid, using phenolphthalein as an indicator. In the case of a highly colored wine, after cooling the liquid make up to 100 cc, measure out 25 cc, dilute with recently boiled distilled water and titrate as before. Express the results for volatile and fixed acids as cubic centimeters of normal acid in 100 cc of the wine.

#### THE DETERMINATION OF GLYCEROL IN WINES.

The method submitted to collaborators has been subjected to trial on a dozen samples of genuine California wines. After the residues obtained by the method had been weighed, they were analyzed for glycerol by the acid-dichromate oxidation method, as follows:

The residue was dissolved in a little distilled water, filtered and washed through a previously dried and weighed small filter, and the solution made up with water to 50 or 100 cc, the volume depending on the amount of the dissolved residue. An aliquot portion of the solution (equivalent to from 0.3 to 0.5 gram of residue) was run into a 200 cc beaker, 20 cc of sulphuric acid (1 : 1) and 50 cc of standard potassium dichromate solution (1 cc equivalent to 0.01 gram of glycerol) run in and the beaker placed in boiling water. During the heating the strength of a prepared solution of ferrous-ammonium sulphate (240 grams in 1,000 cc) was determined by titration with the dichromate. At the end of two hours the beaker was removed from the boiling water, 100 cc of water added, and the excess of dichromate titrated with the ferrous-ammonium sulphate. From the result of the titration the weight of the oxidized glycerol was calculated.

A sample of chemically pure glycerol, which by specific gravity determination and refractometer reading was shown to be 99.3 per cent pure, gave by this method 97.7 per cent pure glycerol by weight. The filter containing the residue insoluble in water was again dried at 100° C., cooled in a desiccator and weighed. Tannin was determined in another aliquot portion of the solution by the official provisional method given for tannin in wine. The results of these determinations are shown in the accompanying table. In three instances, owing to insufficient material, no results for tannin were obtained.

There seem to be no means of estimating the loss of glycerol which takes place during the determination. It appears, however, that the material which is extracted and weighed as glycerol is never pure glycerol, as is generally assumed. The proportion of glycerol obtained by the method of oxidation ranges from 84.7 to 88.5 per cent of the weighed residues. Also it appears that in some cases a considerable amount of the residue consists of matter insoluble in water, and also tannin.

Mr. C. S. Ash, chemist of the California Wine Association, recognizes the need of an improved method for the determination of glycerol, and describes the following, which has been employed in his laboratory:

Measure out 100 cc of wine in a porcelain dish, evaporate to a thick sirup, then make alkaline with milk of lime and evaporate almost to dryness. Dissolve out the glycerol with successive portions of boiling hot alcohol, evaporate the alcohol to about 5 cc, transfer to a stoppered flask or cylinder of 100 cc capacity, make up to 100 cc with acetic ether, and allow to stand over night. Filter the liquid from the precipitate, evaporate off, and dry the glycerol to constant weight at a temperature not above 55° or 60° C.

The method is said to give fairly uniform results, and the most unsatisfactory part of the procedure is the evaporation of the solvent. A small percentage of glycerol is undoubtedly lost, especially toward the end of the process, but this and other difficulties, it is hoped, may be overcome in great measure.

*A comparison of two methods for the determination of glycerol.*

Kind of wine.	Glycerol (in 100 cc).		Water-insoluble residue in extract from 100 cc of wine.	Tannin in extract from 100 cc of wine.	Percentage composition of extract weighed as glycerol in provisional method.			
	By provisional method.	By oxidation method.			Glycerol by oxidation.	Insoluble residue.	Tannin.	Undetermined residue.
	Grams.	Grams.	Grams.	Grams.	Per cent.	Per cent.	Per cent.	Per cent.
Zinfandel.....	0.6974	0.6128	0.0066	0.0115	87.86	0.95	1.65	9.54
Burgundy.....	.2592	.2226	.0016	.0083	85.88	.62	3.20	10.30
Sherry.....	.9509	.8394	.0059	.....	88.48	.61	.....	10.91
Port.....	1.1595	1.0064	.0036	.0400	86.79	.40	3.79	9.02
Muscat.....	1.0424	.8900	.0019	.0325	85.38	.18	3.12	11.32
Hock.....	.6947	.6014	.0037	.0010	86.57	.53	.14	12.76
White wine.....	.6400	.5458	.0010	.....	84.75	.16	.....	15.09
White wine.....	.6008	.5498	.0000	.....	85.79	.00	.....	14.21
Port.....	.6092	.5890	.0012	.0135	88.01	.18	2.02	9.79
Sherry.....	.7765	.6960	.0015	.0210	89.43	.19	2.70	7.48
Claret.....	.7310	.6214	.0033	.0053	85.00	.35	.72	13.83
Angelica.....	.6870	.5856	.0014	.0273	85.24	.20	3.97	10.59

THE DETERMINATION OF REDUCING SUGARS IN WINE.

The following criticism of the provisional volumetric method of the association was prepared by R. M. West, St. Paul, Minn.:

The chief objection to the present provisional methods for the determination of reducing sugars in wine (Bul. 107, p. 87) is the length of time necessary for the operation. Many attempts have been made to remedy this defect by the introduction of volumetric methods, which as a rule have been unsatisfactory either through the necessity of preliminary titrations or through the excessive errors caused by the variable conditions under which the copper is precipitated. It was decided after careful consideration that the actual precipitation of the copper by the provisional method could not be modified to advantage, but that the ordinary methods of preparing the sample, in addition to being long and tedious in operation, are the sources of several serious errors. A strong solution of alcohol fails to reduce Fehling solution, and, such being the case, it is apparent that the addition of 25 cc of a 15 per cent solution of alcohol to 120 cc of boiling diluted Fehling solution, from which it would be almost immediately boiled off, could have little or no influence on the precipitation of the copper. Since the provisional method calls for the dealcoholization of the wine previous to clarification, its omission would save at least a half hour without decreasing the accuracy of the result. Furthermore, it has long been realized that the use of lead subacetate as a clarifying agent has been attended with several errors. In the first place, the precipitated solids carry with them small amounts of sugar, and, in the second place, in taking an aliquot portion of the filtrate the volume of the precipitate is not considered. This second error is repeated when the excess of lead is removed with sodium sulphate, and all these errors together, when multiplied by the number of times that the sample has been diluted during the preparation of the solution in which the sugar is determined, may amount to a considerable proportion of the total reducing sugar content of the wine.

It is proposed, then, that the preliminary treatment of the wine consist of only the dilution necessary to obtain a solution containing not more than 1 per cent of reducing sugar, that the copper be precipitated in the usual way, filtered as quickly as possible, redissolved, and determined volumetrically or by electrolytic deposition. Analyses were made on fourteen samples of wine by both the provisional and the proposed new methods, with the results shown in the accompanying table. To make these results more easily comparable, the sample was diluted with the same amount of water for both determinations.

*Comparison of methods for the determination of reducing sugars in wines.*

Kind of wine.	Total solids in 100 cc.	Reducing sugar in 25 cc of diluted solution (containing not more than 1 per cent of reducing sugar).		Times diluted.	Reducing sugar in 100 cc of wine.	
		By provisional method.	By proposed method.		By provisional method.	By proposed method.
	Grams.	Mg.	Mg.		Grams.	Grams.
Red wine.....	2.40	32.6	31.6	4	0.1304	0.1264
Red wine.....	2.17	27.0	28.1	4	.1080	.1124
Red wine.....	2.18	66.9	62.1	4	.2736	.2484
Sherry.....	4.37	145.9	146.5	16	2.3344	2.3440
Sherry.....	4.77	164.6	162.5	16	2.6336	2.6000
Port.....	12.00	201.9	201.5	40	8.0760	8.0600
Muscate.....	16.22	169.5	169.7	80	13.5600	13.5760
White wine.....	1.72	25.8	26.4	4	.1032	.1056
White wine.....	1.79	21.0	19.4	4	.0840	.0776
White wine.....	1.61	22.5	18.9	4	.0900	.0756
Port.....	13.08	132.2	130.3	80	10.5760	10.4240
Sherry.....	5.47	87.2	85.7	40	3.4880	3.4280
Claret.....	2.34	51.8	59.1	4	.2072	.2304
Angelica.....	12.83	126.5	127.9	80	10.1200	10.2320

EXAMINATION OF THE NATURAL COLORING MATTER IN WINE.

The following statement was contributed by Genevieve Imus, St. Paul, Minn. A complete tabulation of results of tests made on wine colors is given in the Twelfth Biennial Report of the Minnesota State Dairy and Food Department, pages 250 to 253.

During the past two years a considerable amount of time has been given to the examination of the natural coloring matter of wine. The work has not included the detection of coal-tar dyes or other added colors but was intended primarily for the purpose of obtaining on samples of known purity data that would be of value as criteria in future routine analyses. The plan of making all color comparisons and descriptions with reference to reliable standards has been adopted, and to this purpose the color standards employed by Mulliken in his book entitled "A Method for the Identification of Pure Organic Compounds," have been found to be admirably suited. These standards consist of 18 pure colors, and of derived tones, 36 tints, 36 shades, and 12 medium broken colors. They are mounted on cardboard in compact form to facilitate their use in the laboratory. A description of the standards and a discussion of the application of color reactions to the examination of unknown substances are given by Mulliken on pages 230 to 234. In matching colors the best results are obtained if the operator stands with his back to a window and not in direct sunlight; The material to be examined should be held about an inch away from the white cardboard accompanying the color charts and alongside the square opening. The cardboard is moved about until the exposed color matches as nearly as possible the color of the material. A clear day is necessary for satisfactory results.

The tests which are described below were made upon the undiluted wine unless otherwise stated. In tests with the various reagents the resulting colors of the solution have been noted as well as the color of any precipitate which may have been formed. In the solubility tests with amyl alcohol the colors of both the alcohol and resulting wine have been matched on the color chart and recorded.

(a) About 5 cc of the sample are poured into each of six test tubes and the following solubility tests are applied: To each of two portions, one acidified with a few drops of hydrochloric acid and one made alkaline with ammonia, 5 cc of ether are added. To each of two portions, made, respectively, acid and alkaline in the same manner,

5 cc of amyl alcohol are added. The tubes are thoroughly shaken and the liquids added to separate. If in any case an emulsion is formed, a few drops of ethyl alcohol are added. Similar tests are made on the remaining portions of the sample without the addition of acid or alkali.

(b) Ten cubic centimeters of the sample are made alkaline with baryta water, shaken with an equal volume of amyl alcohol and, after observing the colors of the two layers as directed, acetic acid is added to a filtered portion of the amyl alcohol layer.

(c) To 50 cc of the wine in a beaker an equal amount of water and a few cubic centimeters of dilute hydrochloric acid are added. In this is placed a piece of white, fat-free wool cloth, about 10 cm square, and the solution is boiled from five to ten minutes. The cloth is removed, washed in a stream of water, and after noting the color the wool is treated with a 2 per cent ammonia solution.

(d) Five cubic centimeters of concentrated nitric acid are added to an equal amount of the wine in a test tube.

(e) To 10 cc of the sample in a test tube 5 cc of a neutral or slightly alkaline mixture of a 10 per cent potassium alum solution and a 10 per cent sodium carbonate solution are added, the mixture is shaken and the precipitate formed is separated by filtering. In like manner the wine is tested with 5 cc of a mixture of 10 per cent aluminum acetate solution made alkaline with 10 per cent sodium carbonate solution. A portion of the sample is also treated with a 10 per cent solution of mercuric chlorid.

(f) To 15 cc of the sample in a test tube are added 3 cc of a 10 per cent solution of lead subacetate. The solution is shaken and filtered. It has been found difficult at times to match the color of the precipitate closely with the color chart and in such cases the color has been designated in general terms such as blue-gray or brown-gray.

(g) About 0.05 gram of pulverized yellow oxid of mercury is added to 20 cc of the sample and the mixture heated to boiling and poured through a double filter. After first noting the color of the filtrate a few drops of hydrochloric acid are added.

(h) To each of two 10 cc portions of the sample in test tubes a few drops of 10 per cent solutions of ferric chlorid and of ferrous sulphate are added, respectively.

Application of the solubility tests described above has shown the natural color in wines to be insoluble in ether under all conditions and in amyl alcohol when the wine is previously made alkaline with ammonia. A second dye was obtained with one wine, but its dull appearance and its reaction with ammonia afforded a ready means of distinguishing the dye from those of coal-tar origin and from vegetable dyes of the lichen group. Hydrochloric, sulphuric, and acetic acids brighten or intensify the color of the original wine, ammonium and sodium hydroxids darken the solution, and ammonia alum produces no change. Tests with chalk steeped in albumen<sup>a</sup> gave unsatisfactory results.

In general the following conclusions may be drawn from the results obtained by these tests:

1. Nitric acid darkens the original solution of red wines, but produces practically no change in white wines.
2. The lead subacetate precipitates vary from a pale yellow in white wines to a deep blue-gray in red wines, but a violet or red color is never found in the precipitate from a genuine wine.
3. Some highly colored red wines give a slight coloration upon the addition of hydrochloric acid to the filtrate from the yellow oxid of mercury.
4. Ferric salts give a precipitate, ferrous salts do not.

The following results were obtained by H. V. Frost on a sample of red wine labeled "Vino Vecchio del Chianti, Toscano, Italia." No evidence as to the authenticity of the wine was secured, although it was known that the sample was imported from Italy. The designations as to color correspond to those in Mulliken's chart.

<sup>a</sup> U. S. Dept. Agr., Bureau of Chemistry, Cir. 25, p. 17.

## Color reactions using different solvents under varying conditions (Frost).

## GROUP A.

5 cc wine shaken with—	5 cc solvent.		Hydrochloric acid + 5 cc solvent.		Ammonium hydroxid + 5 cc solvent.	
	Upper layer.	Lower layer.	Upper layer.	Lower layer.	Upper layer.	Lower layer.
Ether.....	Colorless..	R-NT.....	Colorless..	R-T1.....	Colorless..	Black.
Amyl alcohol.....	NR-T2.....	R-NT.....	R-T1.....	R-NT.....	Colorless..	Black.
Chloroform.....	R-S1.....	Colorless..	R-NT.....	Colorless..	Black.....	Colorless.
Petroleum ether.....	Colorless..	R-S1.....	Colorless..	R-NT.....	Colorless..	Blackish.
Carbon bisulphid.....	R-S1.....	Colorless..	R-NT.....	Colorless..	Blackish..	Colorless.

## GROUP B.

Determination.	15 cc wine shaken with 3 cc of 10 per cent solution of lead acetate.	10 cc wine shaken with 5 cc slightly alkaline mixture of 10 per cent potassium alum solution and 10 per cent sodium carbonate.	20 cc wine boiled with 0.5 gram pulverized yellow oxid of mercury.
Color of wet precipitate.....	B-BTM.....	BG-S2.....	R-T1.
Color of filtrate.....	VR-T2.....	BG-BTM.....	

## GROUP C.

A piece of fat-free white wool cloth, 10 cm square, was boiled five to ten minutes in 50 cc of wine to which were added 50 cc water and a few cubic centimeters of dilute hydrochloric acid. The cloth was removed and washed in a stream of water. The dyed cloth matched OR-T2; treated with strong ammonium hydroxid, it became yellow-green, matching Y-BTM.

## THE DETERMINATION OF TOTAL, FIXED, AND VOLATILE ACIDS IN WINES.

The first methods of determining the volatile acids in wines consisted in distilling a measured quantity of the sample to about one-third of its original volume and titrating the distillate; the results must obviously have been too low. The next step appears to have been in favor of an indirect course of procedure whereby the total acids were first titrated, then another portion of the wine evaporated off and the fixed acids titrated in the residue. From the difference between these titrations was calculated the amount of volatile acids. Various modifications of this method came into use,<sup>a</sup> in all of which the aim appears to have been to liberate the total volatile acids by a prolonged heating of the extract. That an appreciable change might occur in the extract constituents as a result of such treatment was not at first conceived. At a later date, however, there developed grounds for the belief that certain of the fixed acids disappeared, in consequence of which on titrating the residue there was obtained too small a result; hence the result for volatile acids would be too high. These considerations finally resulted in the abandonment of the so-called indirect methods, and it was again proposed to separate the volatile from the fixed acids by means of distillation and to titrate the volatile acids in the distillate.<sup>a</sup> But because the volatile acids pass over only slowly and with difficulty, a simple distillation, as in the determination of alcohol, could obviously not be employed. Following a number of attempts which had been made to devise a successful method, Lindemann,<sup>b</sup> in

<sup>a</sup> Methods proposed by Kissel, Weigert, Nessler and Barth, and Wolff: Zts. anal. Chem., 1869, 8: 416; 1879, 18: 208; 1883, 22: 166; Repert. anal. Chem., 1883, 1: 213.

<sup>b</sup> Zts. anal. Chem., 1883, 22: 516.



1883, described a method of driving out the volatile acids with steam, and this forms the basis of the official methods which for some years have been prescribed in Europe and in America. In essential details the present official methods<sup>a</sup> appear to comply with the following procedure:

Fifty cubic centimeters of wine are distilled in a current of steam, in the meantime heating the flask containing the sample until the liquid boils, and regulating the flame so that the volume remains constant. Two hundred cubic centimeters of distillate are collected and titrated with tenth-normal sodium hydroxid, using phenolphthalein as indicator.

Chemists who have had considerable experience with this method must have noticed that it often happens that distillates collected beyond 200 cc show a more or less strong acid reaction. This is the case especially with certain red wines, notably burgundys, ports, and clarets, and wines of the sauterne type. In fact, as the accompanying table shows, it seldom if ever occurs that the first 200 cc distillate contains even a fair approximation of the total volatile acids. In some instances it is seen that by carrying the distillation beyond 200 cc the error is very considerable. For example, following closely the provisional method and carrying the distillation to 400 cc, there are shown the following rates of increase in total volatile acids: In a burgundy, approximately 14 per cent; in two samples of port, respectively, 13 and 14 per cent; in a claret, 13 per cent; and in a white wine, 9 per cent. There occur, indeed, wines in which the volatile acids appear never to become completely exhausted, in which, in fact, the distillate fails to appear permanently neutral even after very prolonged distilling. This phenomenon may be attributed not so much to acids of difficult volatility as to a possible decomposition of the extract constituents under the influence of prolonged heating by the direct action of the flame which the official directions require should be maintained below the flask containing the sample. It would seem, therefore, that 300 cc, or at most 400 cc, of distillate should contain practically all of the volatile acids, and that it may not be necessary or practical to prolong the distillation until the last portions of the distillate are neutral.

*Volatile acids in wines (by the present provisional method).*

No.	Kind of wine.	Distillates (cubic centimeters tenth-normal sodium hydroxid required to neutralize).									Acid in first 300 cc.
		First 100 cc.	Second 100 cc.	Third 100 cc.	Fourth 100 cc.	Fifth 100 cc.	First 200 cc.	First 300 cc.	First 400 cc.	Total 500 cc.	
1	Claret.....	6.30	2.10	0.60	0.30	0.15	8.40	9.00	9.30	9.45	<i>Per ct.</i>
2	Zinfandel.....	5.80	1.70	.50	.25	.15	7.50	8.00	8.25	8.40	95.2
3	Burgundy.....	9.25	3.65	1.25	.55	.20	12.90	14.15	14.70	14.90	95.0
4	Sherry.....	4.65	1.55	.50	.25	.10	6.20	6.70	6.95	7.05	93.6
5	Sherry.....	5.60	2.20	1.10	.70	.30	7.80	8.90	9.60	9.90	90.0
6	Port.....	10.70	3.45	1.25	.65	.25	14.15	15.40	16.05	16.30	94.7
7	Port.....	5.45	2.00	.70	.35	.15	7.45	8.15	8.50	8.65	94.2
8	Muscat.....	2.75	1.00	.40	.25	.10	3.75	4.15	4.40	4.50	92.2
9	Hoek.....	2.85	.90	.50	.25	.10	3.75	4.25	4.50	4.60	92.4
10	White wine.....	4.45	1.45	.80	.45	.15	5.90	6.70	7.15	7.30	91.7
11	White wine.....	6.90	2.25	.50	.35	.20	9.15	9.65	10.00	10.20	94.6
12	Port.....	2.15	.80	.35	.20	.00	2.95	3.30	3.50	3.50	94.3
13	Sherry.....	5.90	2.40	.85	.45	.15	8.30	9.15	9.60	9.75	93.8
14	Claret.....	7.65	2.65	.95	.40	.15	10.30	11.25	11.65	11.80	95.7
15	Angelica.....	4.70	1.65	.60	.30	.10	6.35	6.95	7.25	7.35	94.5
16	Port.....	4.10	1.95	.75	.35	.15	6.05	6.80	7.15	7.30	93.1

<sup>a</sup> First 11 samples furnished by California Wine Association, 1906.

<sup>b</sup> Last 5 samples from miscellaneous sources.

The results obtained on these 16 samples of wine show that in only four cases did the fifth 100 cc distillate require as much as 0.2 cc of tenth-normal alkali to neutralize; hence, for practical purposes, it may be assumed that the vanishing point of the

volatile acids occurs when 500 cc of distillate have passed over. On this basis the results show that the proportion of volatile acids collected in the first 300 cc of distillate ranges from 90 to 95.7 per cent, only four samples showing a proportion slightly greater than 95 per cent. Thus, even in the present provisional method of the association, if the entire apparatus be of fixed dimensions and relations in addition to the other conditions stipulated, a large part of the fundamental error is still retained even by carrying the distillation to 300 cc. If the distillation be conducted slowly and the volume of the wine permitted to become too large, an insufficient amount of volatile acids will pass over; if the distillation be too rapid and the volume of wine be permitted to diminish too much, there may occur an overheating and the amount of volatile acids will be too great. Finally, in order to obtain the greatest possible concordance in results, the distillation must be watched from beginning to end with the greatest care.

It has also been recognized that the agreement in results obtained by several distillations on a given sample fails to reach as high a degree as ought to be expected, even in approximately exact determinations. When the distillations are carried to 400 cc, or even until the vanishing point of acidity is fairly reached, the results often fail to agree within reasonable limits. A difference in the results of the titrations amounting to from 0.3 to 0.5 cc of tenth-normal alkali has often been noted; and the differences are commonly far greater at the close of the 200 cc period, amounting to from 0.6 to 0.8 cc in several instances.

Briefly, then, the objections to the present provisional method are:

(1) The method is complicated, requiring rather elaborate apparatus and tiresome supervision.

(2) The prolonged heating of the wine by the direct action of the flame doubtless affects in some manner the constitution of the acid ingredients.

(3) The results do not to a sufficient extent represent the total volatile acids.

(4) The results are not reasonably concordant in the hands of different persons or even in the hands of a single individual.

Owing to these considerations, various chemists have proposed the abandonment of the direct method of determining volatile acids in favor of an indirect course of procedure. After a prolonged examination of the relative merits of these two general methods, Windisch <sup>a</sup> proposes the following:

Twenty-five cubic centimeters of wine are titrated in the usual manner for total acids, using litmus or litmus paper as indicator. Another 25 cc portion is then evaporated on a water-bath in a porcelain dish to 3 to 5 cc, the residue dissolved in about 25 cc of hot water, the liquor again evaporated to 3 to 5 cc, the residue again dissolved in about 25 cc of hot water, and the liquor evaporated a third time to 3 to 5 cc. Finally, the residue is dissolved in hot water and the fixed acids titrated, using litmus as an indicator. From the difference between these titrations the volatile acids are calculated:

The advantages of this over the present provisional direct method are obvious, and have been adequately demonstrated by Windisch and others. The wine is never heated above the temperature of the water-bath and the volatile acids are undoubtedly all driven out, leaving the fixed acids probably unchanged. Furthermore, the results appear to be reasonably concordant and satisfactory. Various modifications of the indirect method of obtaining the volatile acids have appeared.<sup>b</sup>

Sellier <sup>c</sup> has described a simple apparatus which consists of a small wide-neck flask into which is fitted a cylindrical-shaped flask. In the bottom of the latter flask is a

<sup>a</sup> Zts. Nahr. Genussm., 1905, 9: 70.

<sup>b</sup> Methods proposed by Roos and Mestrezat, Guerin, Curtel, and Robin: Bul. assoc. chim. suc., 1907, 25: 41-49; J. pharm. chim., 1907, 25: 491-492; Ann. chim. anal., 1901, 6: 361; J. pharm. chim., 1904, 19: 531-533.

<sup>c</sup> Ann. chim. anal., 1901, 6: 414.

small opening leading inward through a siphon-shaped tube bent back upon itself and terminating at a point close to the bottom. In making the determination, 50 to 60 cc of distilled water are placed in the larger flask, the smaller flask fitted into the wide neck by means of a section of rubber tubing, 10 cc of wine run in and the water heated to boiling. The steam passing through the siphon-tube and through the wine carries out the volatile acids. No appreciable change in the volume of the wine takes place. When the water is reduced to about 5 cc, the flame is removed. On cooling the apparatus the remaining wine liquor is drawn down into the larger flask. The small flask is rinsed out with a little hot water and the two flasks disconnected. The liquor is cooled and the fixed acids are titrated.

This method has been employed in the laboratory of the Minnesota Dairy and Food Department in the analysis of a number of samples of wine and in the investigation of a dozen or more of the common varieties of fruit juices, and has proven satisfactory not only from the standpoint of convenience in manipulation but on account of the fact that results appear to be reliable and concordant. It has been noted, however, that in this method as in others the volatile acids are not collected but are allowed to dissipate into the air, and it has seemed desirable to condense the vapors and titrate the volatile acids in the distillate. By joining a condenser to the flask containing the sample there is provided an apparatus (see fig. 1) whereby may be determined in one operation both the volatile and fixed acids on the same portion of wine.

The statement of the method proposed for total, volatile, and fixed acids is given on page 13.

In the laboratory of the California Wine Association the following method of titration is employed:<sup>a</sup>

Ten cubic centimeters of wine are measured into a 500 cc beaker without the addition of water. The wine is well shaken to remove carbon dioxide and titrated directly with fifth-normal sodium hydroxide. In the case of heavy-colored wines, no indicator is used; the coloring matter of the wine indicates the end point of the titration. In the case of white wines, the same method of procedure is followed excepting that a little neutral litmus is added. In titrating light-colored red wines, it may be advisable to add litmus, but the indicator is never used unless absolutely required.

A comparison of the results obtained by the various methods of determining total, fixed, and volatile acids is shown in the accompanying table. Total acids were determined by the California Wine Association method, by the method of Windisch and by the proposed new method based on that given by Breteau. Removal of carbonic acid was assured before any of the methods were attempted. Fixed acids were determined by the method of Windisch, by the method of Sellier, and by the proposed new

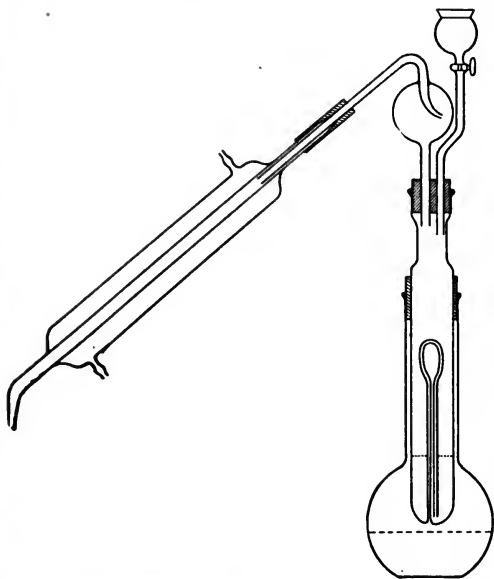


FIG. 1.—Apparatus for determining volatile and fixed acids in wine.

<sup>a</sup> According to letter received from C. S. Ash, chemist, California Wine Association.

method of titration after driving off the volatile acids by steam distillation. Volatile acids were determined by the indirect method of Windisch and by the proposed new direct method of distilling by steam. In the Windisch methods the titrations were made using litmus paper as an indicator and in the method of Sellier, as in the proposed new methods, phenolphthalein was used.

*Comparison of methods for the determination of total, fixed, and volatile acids in wines.*

[Results expressed as cc tenth-normal acid in 100 cc of sample.]

No.	Kind of wine.	Total acids.				Fixed acids.				
		Method of California Wine Association.	Method of Windisch.	Proposed new method.	Calculated from fixed and volatile acids.	Method of Windisch.	Method of Sellier.	Proposed new method.		Calculated from total and volatile acids.
								Using litmus.	Using phenolphthalein.	
1	Angelica.....	4.30	4.16	5.80	5.80	3.32	4.90	3.40	5.00	5.00
2	Claret.....	7.70	7.52	9.60	9.55	6.80	8.00	7.00	8.00	8.05
3	Port.....	5.20	5.60	7.20	7.15	4.36	5.90	5.00	6.00	6.05
4	Riesling.....	6.00	6.36	7.45	7.50	4.80	6.15	5.20	6.10	6.05
5	Sherry.....	4.00	4.50	5.35	5.35	2.96	3.95	3.00	3.90	3.90
a6	Zinfandel.....	8.70	7.92	9.70	9.75	5.64	6.75	5.50	6.75	6.70
b7	Sauterne.....	6.70	7.58	8.55	8.60	5.48	6.40	5.00	6.50	6.45
b8	Bordeaux.....	7.10	8.16	9.15	9.20	6.00	7.30	6.00	6.95	6.90
9	Sauterne.....	7.70	8.20	8.90	8.95	6.00	7.75	6.50	7.30	7.25
10	Port.....	8.90	8.44	9.55	9.60	3.60	4.80	3.70	4.40	4.40
11	Sherry.....	5.50	5.80	6.60	6.60	7.40	9.60	8.20	9.20	9.20
c12	Claret.....	5.60	10.60	11.90	11.90	4.40	5.50	4.50	5.20	5.20
d13	Port.....	6.80	6.30	7.10	7.10	3.60	5.70	4.00	4.80	4.75
14	Sherry.....	5.90	6.40	7.20	7.25	6.20	7.90	6.40	7.70	7.60
15	Claret.....	8.50	8.40	9.50	9.60	6.14	8.20	6.55	8.10	8.05

No.	Kind of wine.	Volatile acids.				Ratio of volatile to fixed acids.	
		Method of Windisch.	Proposed new method.		Calculated from total and fixed acids.	According to method of Windisch.	According to proposed new method.
			Using litmus.	Using phenolphthalein.			
1	Angelica.....	0.84	0.70	0.85	0.80	1 : 3.95	1 : 6.25
2	Claret.....	1.72	1.40	1.55	1.60	1 : 3.95	1 : 5.16
3	Port.....	1.24	.95	1.15	1.20	1 : 3.51	1 : 5.21
4	Riesling.....	1.56	1.15	1.40	1.35	1 : 3.07	1 : 4.35
5	Sherry.....	1.54	1.20	1.45	1.45	1 : 1.92	1 : 2.69
a6	Zinfandel.....	1.94	1.30	1.85	1.80	1 : 2.90	1 : 3.69
b7	Sauterne.....	2.68	2.10	2.70	2.65	1 : 2.04	1 : 2.40
b8	Bordeaux.....	2.20	1.70	2.00	1.95	1 : 2.73	1 : 3.47
9	Sauterne.....	2.44	2.00	2.30	2.25	1 : 2.46	1 : 3.17
10	Port.....	2.20	1.90	2.20	2.20	1 : 1.64	1 : 2.00
11	Sherry.....	3.20	2.40	2.70	2.70	1 : 2.31	1 : 3.41
c12	Claret.....	1.90	1.60	1.90	1.90	1 : 2.32	1 : 2.74
d13	Port.....	2.80	2.00	2.45	2.40	1 : 1.28	1 : 1.96
14	Sherry.....	2.20	1.60	1.90	1.80	1 : 2.82	1 : 4.05
15	Claret.....	1.78	1.40	1.65	1.60	1 : 3.44	1 : 5.06

a First six wines furnished by the California Wine Association, 1908.

b 7 and 8 obtained from local dealers, 1908, St. Paul, Minn.

c 9 to 12, inclusive, from a Rochester, N. Y., wine company, 1908.

d 13 to 15, inclusive, from a Norfolk, Va., wine company, 1908.

The results obtained by the California Wine Association method were not satisfactory, the end-point of the titrations being, in most instances, very uncertain. In the angelicas, ports, and sherrys especially, much difficulty was experienced

in carrying out the titrations, and the results were scarcely better when litmus tincture was used. In titrating according to the Windisch methods the point of neutrality was judged to be attained when a small drop of the liquor placed on delicate blue litmus paper just ceased to produce a perceptible red. There appeared to be decided disadvantages in using litmus paper, and the use of litmus tincture even in a clear distillate is open to serious objections, which will be stated presently. In colored wines especially the difficulties were very great, and it was found well-nigh impossible at times to devise a means whereby to judge with reasonable certainty the true end-point of the titration. It was found, however, after considerable practice, that fairly concordant results were obtainable by this method in the majority of instances. Phenolphthalein, on the other hand, while not entirely unobjectionable, was found to give far greater satisfaction. While it was not always convenient to titrate on the undiluted sample, especially in the case of wines containing more or less natural coloring matter, it was found to be entirely permissible, as in the titration of cider vinegars, to dilute with boiled distilled water in order to carry out a successful titration with phenolphthalein. It has been shown that the end-point of a titration can be very accurately judged, even in a deeply-colored wine, and that the addition of water to the extent of 100 or 200 cc does not introduce a serious error in the result. As in a cider vinegar, the change in the color of a wine occurs at a much earlier stage than the change in the indicator and there is never a serious difficulty in safely judging the end point.

As already pointed out, the results shown in the first column of figures are at best only rough approximations. In the majority of instances it was observed that when litmus paper was used the titrations were carried somewhat beyond the point of neutrality which seemed to be indicated by the change in the natural coloring matter of the wine. It is also noted that the results obtained by the titrations employing litmus were uniformly much lower than the results obtained with phenolphthalein. This is true not only in the titrations of total and fixed acids, but also in the direct titrations of the volatile acids. On the basis of the results obtained with phenolphthalein, litmus indicates approximately from 77 to 92 per cent of the total acids and from 58 to 85 per cent of the fixed acids. Doubtless there are theoretical reasons underlying these differences, and the question may well be raised as to whether chemists have given due attention to these considerations in choosing indicators for titrating the acids in wines.

In the first place, there appears to be little justification for the practice adopted by some chemists of employing the natural coloring matter as a correct indicator in titrating either the total or fixed acids. Little of value is known regarding the action of the oenocyanin or other coloring substances in the presence of acids or alkalis, and it is certain that such substances have not been recommended in the titration of any of the common acids. In the case of litmus also there are some important considerations which should bar it as an indicator for wines as well as fruit products in general. Litmus is not recommended for titrating such acids as tartaric, acetic, tannic, succinic, or malic. In titrating tartaric acid with this indicator, the change is gradual and the end-point indistinct, while in titrating acetic acid, the acetate of sodium formed is alkaline to litmus and tends strongly to hasten the end-point. On titrating solutions of tannic acid, a change takes place almost immediately on beginning the titration, and only a small proportion of the actual acid is indicated. Phenolphthalein, on the other hand, is a very satisfactory indicator with all these acids, and, with the exception of tannic acid, the theoretical amount of acid is obtained. About 80 per cent of tannic acid is indicated, but the total acid is obtained after boiling with a measured small amount of tenth-normal hydrochloric acid.

As a means of shedding some light on the differences occurring in titrating wines with the two indicators, the determinations shown in the following table have been carried out:

*Comparison of litmus and phenolphthalein as indicators in titrating some of the organic acids existing in wines.*

Acid.	Description.	Grams in 100 cc.	Normal acid in 100 cc.			Per cent acid indicated.	
			Calculated.	Using phenolphthalein.	Using litmus.	Using phenolphthalein.	Using litmus.
Tartaric.....	Elmer and Amend.....	0.4000	cc 5.33	cc 5.35	cc 5.10	100.3	95.6
Acetic.....	Mallinckrodt's 99 per cent.....	.4040	6.67	6.70	6.35	100.4	95.2
Potassium bitartrate.....	Elmer and Amend.....	.4000	2.12	2.10	1.90	99.0	89.6
Tannic.....	J. T. Baker Chemical Co.....	.5000	2.79	2.20	.40	78.8	14.3
Tannic.....	After boiling with dilute hydrochloric acid.	.....	.....	2.80	.40	100.3	14.3
Average (omitting last item).	.....	.....	.....	.....	.....	94.4	73.6

The results shown for volatile acids by the Windisch method (p. 22) are somewhat higher than those obtained by the proposed new method, using phenolphthalein. Such discrepancies, however, lose their significance when it is considered that in the determination of volatile acids by the indirect method not only are the results of the titrations employing litmus as indicator incorrect, but the titrations of total and fixed acids are not made under comparable conditions. While it is unquestionably true that the volatile acids may be completely driven off by repeated evaporation in an open dish, it does not follow that the results obtained by means of the two titrations are correct. It is conceivable that important changes may occur during the prolonged heating of the wine in order to reduce the material a third time to a pasty consistency. At any rate, we have no positive knowledge that the so-called fixed acids occurring in the final residue represent the actual fixed acids in the original wine. A titration of the residue may suffice as an indication of the acids remaining after driving off the volatile constituents by prolonged heating, but to employ the result of such a titration as a factor in the calculation of the actual volatile acids appears to be an unwarranted proceeding.

In expressing results of analysis the orthodox custom appears to be to calculate the fixed and total acids as tartaric and the volatile acids as acetic. It is impossible to concede any advantages in favor of this custom. It may be safe to assume that in wines the fixed acids are in the main tartaric and the volatile acids acetic; but, even on such assumptions, the results are strictly erroneous and not readily comprehended. Such a method applied to the various fruit juices and ciders would fail to give significant results in practically all cases, and the case is still worse when one adopts the method of calculating the acids as sulphuric. Instead of these conventional methods it has been found better to adopt the plan of expressing all results for total, volatile, and fixed acids in terms of the number of cubic centimeters of normal acid in a definite measure, say 100 cc, of wine. There will then be afforded results which can be readily compared and comprehended. Furthermore, in case it be required to calculate results in terms of any particular acid, such an operation can easily be carried out.

#### RECOMMENDATIONS.

(1) The standard temperature for the determination of specific gravity should be changed to 20°C. A statement of reasons for this change seems to be unnecessary, as the matter has been fully discussed by others, and many chemists have for some time

adopted the custom of making determinations at a temperature not far from that of the average laboratory. If the alcohol tables can be revised in accordance with a standard temperature of 20° C. for specific gravity determinations, a very useful service will be performed, especially in the interest of industrial and food chemists.

(2) The method for glycerol should be made a subject for special study. Experience has shown that it is possible not only to increase the accuracy of the method but to shorten the time of the operation. As the provisional method now stands, it appears to be rather tedious, and there are too many opportunities for error. A large error undoubtedly occurs during the evaporations as well as during the repeated extractions. Also, it appears that the residue weighed as glycerol is far from being pure.

(3) The present methods for determining total, fixed, and volatile acids are exceedingly faulty. The method for volatile acids, especially, fails to give results anywhere near the truth. The difficulty lies not only in the collection of 200 cc distillate but in the operation, which is cumbersome and unreliable. The use of litmus in the titrations of total and fixed acids is open to criticism, as that indicator fails to show all of the acids. A study of the proposed new methods is recommended.

(4) A more comprehensive scheme for the examination of the natural coloring matter of wines is required. Attention is called to the use of standard color charts as a means of obtaining comparable results in the hands of different persons. It is recommended that the association make a special study of the character and properties of the coloring matters existing in genuine wines.

#### REPORT ON BEER.

By H. E. BARNARD, *Associate Referee.*

Mr. Barnard, referee on beer, reported that no cooperative work on the subject had been done, and made the following statement in regard to the condition of the methods:

Two years ago I presented beer methods which have since been adopted as provisional. I have been working with those methods since that time and find no special necessity for changing them. For that reason I have not made a special report on beer. Much work, however, seems to be necessary if we must determine the different kinds of beer, and I would only suggest to you the necessity for a careful study of methods of beer analysis with special reference to the adoption of some method which will enable us to tell more accurately than is at present possible whether or not beer is brewed from all malt, or part malt, or from malt substitutes.

#### REPORT ON DISTILLED LIQUORS: COOPERATIVE TEST OF METHODS FOR THE DETERMINATION OF FUSEL OIL.

By L. M. TOLMAN, *Associate Referee.*

The cooperative work undertaken this year was a comparison of the present Allen-Marquardt method, as given in Bulletin 107, revised, page 98, and a proposed modification worked out by the associate referee and his assistants. The modification was based on the determination of the amount of bichromate reduced in the oxidation of the higher alcohols. This method eliminates the distillation of the acids, which the experiments made have shown are not completely distilled off. In order to test this modified method (for details see paper, p. 206) a series of samples was prepared containing varying amounts of pure amyl alcohol (boiling point 131° C.) in approximately 50 per cent by volume ethyl alcohol, and the samples sent to eighteen different laboratories, asking for a comparison of the modified method with the present method as given in Bulletin 107. Eleven reports were received, and the following table gives

the results, the percentage yields being calculated from the grams of amyl alcohol per 100,000 of 100-proof alcohol, as determined by each method.

*Comparison of the Allen-Marquardt method and the proposed modification for the determination of fusel oil, using varying amounts of amyl alcohol.*

Collaborator.	0.050 gram.		0.100 gram.		0.150 gram.		0.200 gram.		0.250 gram.		0.350 gram.		
	Allen-Marquardt method.	Modification.	Allen-Marquardt method.	Modification.	Allen-Marquardt method.	Modification.	Allen-Marquardt method.	Modification.	Allen-Marquardt method.	Modification.	Allen-Marquardt method.	Modification.	
Detroit laboratory.....	P. ct. 99.8	P. ct. 102.0	P. ct. 78.7	P. ct. 105.0	P. ct. 110.0	P. ct. 130.4	P. ct. 74.1	P. ct. 78.34	P. ct. 62.7	P. ct. 77.74	P. ct. 97.69	P. ct. 100.0	P. ct. 85.14
New York laboratory.....	100.0	122.0	82.0	113.0	72.11	86.0	73.8	85.4	78.5	88.06	74.5	87.7	79.7
Philadelphia laboratory.....	56.0	106.4	99.70	105.1	80.4	96.6	83.4	95.02	79.4	104.0	104.0	76.7	87.24
Portland laboratory.....	120.0	125.9	108.8	104.5	100.0	114.9	87.06	108.6	87.86	85.64	80.6	81.68	81.7
Sunnybrook Distilling Co.	103.0	.....	82.64	.....	.....	.....	78.0	.....	83.22	.....	69.06	.....	87.7
St. Paul laboratory.....	67.3	134.2	72.1	97.1	62.2	91.5	65.2	86.2	68.3	88.5	68.8	84.6	84.6
Washington laboratory.....	87.20	87.24	79.28	82.14	.....	.....	80.0	79.90	81.36	78.60	72.06	75.52	75.52
Galveston laboratory <sup>a</sup> .....	.....	.....	.....	.....	42.8	63.1	50.44	74.2	53.74	80.0	50.9	73.22	73.22
San Francisco laboratory <sup>a</sup> .....	75.6	70.4	.....	.....	.....	.....	61.62	66.60	70.16	62.14	61.86	68.19	68.19
Seattle laboratory <sup>a</sup> .....	84.40	145.7	.....	119.1	76.04	124.5	76.36	131.4	.....	121.0	.....	110.3	110.3
Boston laboratory <sup>a</sup> .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average.....	89.7	117.2	85.7	99.5	85.3	103.7	77.7	94.7	76.2	91.5	76.7	85.8	85.8

<sup>a</sup> Excluded from average.

Some of the results obtained at laboratories which had not had experience in operating the method varied markedly from the other figures and are omitted from the average. The averaged results on the various amounts by both methods are plotted, using as the abscissa of the curves the amount of amyl alcohol used in grams per 100,000 of proof spirit and as ordinates the average percentage yield.

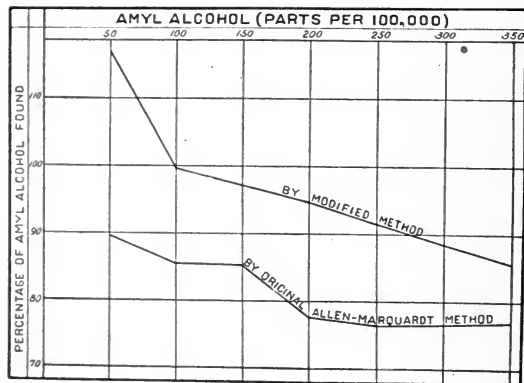


Fig. 2.—Graphic of collaborators' results on amyl alcohol by the Allen-Marquardt method and the proposed modification.

is obtained by carrying the distillation much further, as is pointed out in the supplementary paper submitted on this subject (p. 206). There is also shown a uniformly increasing loss by both methods as the per cents of amyl alcohol increase. This is doubtless due to the method of extraction, as a 100 per cent yield can be obtained in the oxidation part of the method, as was demonstrated in the experimental work on the determination of the factor 0.001773 (see p. 210). From these results it is evident that a higher yield is due to the more correct estimation of the higher alcohols present in the

This curve (fig. 2) shows that the new modification gives uniformly higher results, indicating a regular loss in the old method. This loss is undoubtedly largely due, as is shown by the experiments, to the failure to drive over all of the volatile acids in the distilling method unless a much larger amount of water is distilled than that prescribed in the present provisional method. A very much higher yield of acids



carbon tetrachlorid extract. The curve also shows that the collaborators obtained more uniform results from the new modification than from the method as originally stated. It may be concluded, therefore, that—

- (1) The modified method gives higher and more uniform results.
- (2) It eliminates a tedious and inaccurate distillation.
- (3) It is quicker and gives an opportunity to make check titrations on the same sample.

#### RECOMMENDATIONS.

As a result of this year's work, including that reported on page 206, it is recommended—

- (1) That this modification of the Allen-Marquardt method be adopted as a provisional method (see p. 210).
- (2) That in the present method a second washing with sodium sulphate be prescribed.
- (3) That the method for determining the water-insoluble color be adopted as a provisional method (see p. 207).
- (4) That the method for the determination of amyl insoluble color be adopted as provisional (quantitative Marsh test method, p. 206).
- (5) That the Roese method given in Bulletin 107, page 97, be dropped as a provisional method on account of the entirely unsatisfactory results obtained with it in the past two or three years.

Mr. Tolman called on Mr. Fischer, of the Bureau of Standards, who spoke in regard to the necessity of unifying the alcohol tables. He called attention to the fact that two tables are now in use by the Treasury Department and a third by the Association of Official Agricultural Chemists. The disadvantages to chemists and practical workers from such a condition of affairs being obvious, it was strongly recommended that the association take some action in the matter. A table based on the calculations of Mendeléeff was recommended by Mr. Fischer. The question of temperature was also discussed, and the whole matter was temporarily referred to Committee C on recommendations of referees, the chairman naming the following members to serve on this committee: Messrs. Tolman, Winton, Hortvet, Bartlett, and Jaffa.

#### REPORT ON VINEGAR.

By CHARLES H. HICKEY, *Associate Referee.*

The work which has been done by or reported to the referee deals largely with the lead number for pure cider vinegar and other pure vinegars. The cider vinegars used by the referee were made by the old-fashioned, slow process. The method employed is similar to the one by which Winton and Kreider<sup>a</sup> analyzed maple products, with some modifications to make it applicable to vinegar. The number of grams of lead precipitated by 100 cc of vinegar is taken as the lead number. Other data are included to make the results more complete. The method as modified by the referee is as follows:

Pipette 25 cc of vinegar into a 100 cc flask; add 5 cc of a standard lead subacetate solution and dilute to 100 cc. Let stand at least one hour, then filter and pipette out 50 cc of the clear filtrate. To this add 10 cc of dilute sulphuric acid and 100 cc of 95 per cent alcohol; let stand over night; filter through a porcelain gooch crucible; wash

<sup>a</sup>J. Amer. Chem. Soc., 1906, 28 : 1204.

with 95 per cent alcohol; dry at a moderate heat for a few minutes, cool and weigh. Calculate the amount of lead in the precipitate (factor 0.6829) and subtract this from the amount in 2.5 cc of the standard solution as determined on a blank test, and divide the remainder by 0.125, thus obtaining the lead number.

The standard lead subacetate used in this work is prepared as follows: Dilute a U. S. P. lead subacetate solution until the specific gravity is 1.25; to one part of this add four parts of water and filter. If the solution becomes cloudy, filter before using, and determine its strength frequently. The referee found that the strength changed but little.

Mr. E. M. Bailey of the agricultural experiment station at New Haven, Conn., has reported work which he did independently on different kinds of samples of vinegar of known purity. He includes other data in his results, especially those on testing a recent method for the determination of malic acid which was formerly applied to maple products.<sup>a</sup> He found that by using this method more malic acid can be recovered than by the old calcium chlorid method. The method for determining the lead number is as follows:

Measure 50 cc of vinegar into a 100 cc flask, add 25 cc of lead subacetate (dilute solution used by Winton and Kreider) make up to the mark and filter. To 10 cc of the filtrate add 1 cc of concentrated sulphuric acid, 40 cc of water and 100 cc of 95 per cent alcohol. Filter after 12 hours, ignite, and weigh.

The amount of lead in the blank test is determined by diluting 25 cc of the lead subacetate solution to 100 cc; 10 cc are taken out and the lead number determined as in the method just given.

The modified method for malic acid as applied to vinegar is as follows:

To 10 cc of vinegar add an equal volume of water, 3 cc of a 10 per cent solution of calcium acetate, and 180 cc of 95 per cent alcohol. Heat on the steam bath for from 20 to 30 minutes, stirring vigorously at intervals to insure a clear supernatant liquid. Filter on 589 S and S paper, wash with 85 per cent alcohol, and ignite. Dissolve in excess of tenth-normal hydrochloric acid (10 cc) by gentle boiling, and continue to boil for about 10 minutes. Cool and titrate with tenth-normal sodium hydroxid, using methyl orange as indicator.

The results of the work of the referee and those of Mr. Bailey appear in the accompanying table. This shows the variation in the amount of lead precipitated by the different vinegars. In the case of malt vinegar, the results tend to run high; while those of the sirup and distilled vinegar are very low.

It should be noted that in the case of malic acid determinations, which are, of course, not properly such on malt and sirup vinegar, misleading results may be obtained and, in the case of a suspected sample, the malic acid determination would have to be confirmed by the procedure recommended by Leach and Lythgoe.<sup>b</sup>

In comparing the figures for malic acid, phosphates, and the lead number as worked out by Mr. Bailey, it is his opinion that a closer relation exists between the phosphate content and the lead number than between the malic acid value and the lead number. The three highest lead numbers are associated with the three highest total phosphate values; the same is true of the three lowest figures in each case. That this does not follow, however, in the case of lead numbers and malic acid values would seem to indicate that the precipitate produced on adding lead acetate to vinegar is due rather to the phosphates than to the malates. This is in accordance with the statement of Leach and Lythgoe<sup>c</sup> that the precipitate produced by lead acetate is not entirely due to malic acid. Tolman and Le Clerc<sup>d</sup> are also of this opinion.

The other data for pure cider vinegar, included in the table, are fairly typical, and in addition to the old figures the new ones for the lead number are of interest.

<sup>a</sup>J. Amer. Chem. Soc., 1908, 30 : 1285.

<sup>b</sup>J. Amer. Chem. Soc., 1904, 26 : 379.

<sup>c</sup>J. Amer. Chem. Soc., 1904, 26 : 380.

<sup>d</sup>U. S. Dept. Agr., Bureau of Chemistry, Bul. 99, p. 89.

## Analyses of samples of vinegar of known purity.

C. H. HICKEY.

Number of sample.	Character.	Solids.	Acid.	Ash.	Reducing sugars (dextrose).		Reducing sugars in solids.	Polarization (200 mm Ventzke tube).	Mallic acid.	Phosphoric acid (per 100 cc).		Lead number for 100 cc.
					Direct.	Invert.				Total.	Soluble.	
1	Cider vinegar	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	°V	P. ct.	mg.	mg.	0.166
2	do	1.44	5.16									.134
3	do	2.69	8.70									.087
4	do	1.50	5.06									.145
5	do	1.70	4.57	.38	0.13	0.13	7.6	-1.10				.106
6	do	2.18	4.60	.35	.24	.24	11.4	-1.87				.112
7	do	1.75	5.62	.38	.12	.10	6.3	-1.54				.095
8	do	1.98	5.78	.32	.21	.19	10.1	-0.88				.114
9	do	2.04	6.30	.37	.22	.20	10.2	-1.43				.103
10	do	2.10	7.84	.48	.11	.11	5.2	-1.21				.114
11	do	1.86	6.24	.40	.12	.12	6.5	-0.88				.103
12	do	1.76	5.74	.30	.14	.13	7.7	-1.10				.109
13	do	1.50	3.00	.34	.09	.09	6.0	-0.77				a.098
14	do	1.94	5.80	.46	.14	.18	8.3	-0.88				.129
15	do	1.73	4.84		.16	.16	9.3	-0.88				.106
16	do	1.35	4.60	.32	.10	.10	7.4	-0.88				.087
17	do	1.60	4.72		.12	.11	7.2	-1.10				.163
18	do	1.80	7.28	.40	.13	.11	6.7	-1.08				.076
19	do											.109

E. M. BAILEY.

21	Cider vinegar	1.50							0.073			0.075
22	do	2.52			1.01	1.04	40.0		.129	15.1	10.2	.116
23	do	2.34			.95	.99	41.4		.121	15.9	9.0	.088
24	do	2.34			.50	.53	21.3		.411	16.6	6.7	.148
25	do	2.77			.86	.93	32.5		.296	26.9	13.8	.174
26	do				.87	.89			.198	46.1	39.9	.290
27	do				.62	.64			.221	18.4	10.8	.122
28	do	3.45			1.12	1.14	32.3		.399	30.0	16.9	.220
29	Malt vinegar <sup>b</sup>	2.40	5.74	0.06								.434
30	do	2.79			.90	.97	33.3		.230	106.2	17.8	.548
31	do	1.69			.08	.09	5.3		.120	31.5	14.6	.158
32	Distilled white	0.25			.01	.02	8.0		.016	5.9	3.3	.018
33	Sirup	1.17			.19	.20	17.0		.069	12.8	1.8	.021
34	do. <sup>b</sup>	1.60	4.48									.015

<sup>a</sup> Incompletely aceticified<sup>b</sup> Not analyzed by E. M. Bailey.

## REPORT ON FLAVORING EXTRACTS.

By E. M. CHACE, Associate Referee.

No report on flavoring extracts was submitted by the referee last year, owing to the fact that only a very limited amount of work was done, and the report this year includes also the work submitted by collaborators in 1907.

## WORK OF 1907.

In 1907 the following samples were sent to collaborators, with the usual instructions.<sup>a</sup>

No. 1. Blank containing no lemon oil or citral.

No. 2. Alcohol 1,900 grams, lemon oil 100 grams.

No. 3. Lemon oil used in preparing No. 2.

No. 4. Alcohol 2,000 grams, vanillin 2 grams, coumarin 2 grams, acetanilid 1 gram.

No. 5. Extract prepared from Mexican vanilla beans by the U. S. P. method.

<sup>a</sup> The methods for citral were the same as those reported for 1908, see page 32; for vanilla methods see Bul. 107, p. 156.

Reports were received in all from seven collaborators, and the results are tabulated as follows:

*Citral determinations in lemon extracts and oil, 1907.*

Collaborator.	Sample 1.	Sample 2.	Sample 3.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
A. P. Sy, Washington, D. C. ....	None	0.16	5.23
B. H. Smith, Boston. ....	Trace	.300	6.12
C. S. Brinton, Philadelphia. ....	Not reported	.322	Not reported
A. F. Seeker, New York. ....	0.01	.226	4.61
Do. <sup>a</sup> .....		.209	4.50
L. A. Brown, North Dakota. ....	.00	.229	5.41
W. A. Syme, North Carolina. ....	.027	.126	3.50

<sup>a</sup> Using ice water bath.

COMMENTS OF THE ANALYSTS.

*A. P. Sy:* Sample No. 2—oil globules had separated in original sample. Shook well before making determination.

*B. H. Smith:* These were the first samples personally examined by this method and I have not found time to repeat the work as was intended before reporting results.

*C. S. Brinton, and T. F. Pappé:* We desire to state several points which will probably have a bearing on the value of these results: First, the metaphenylene diamin hydrochlorid at our disposal showed signs of being somewhat decomposed and the aldehyde-free alcohol, as a result, gave a quite marked coloration with the reagent. Second, although we allowed several days to elapse before making up to volume our fuchsin sulphur dioxide reagent, it did not become colorless but showed a deep lemon yellow tint.

*A. F. Seeker:* In laboratories where constant temperature baths are not at hand and when only occasional citral determinations are required, it will be found much more convenient to use ice water for immersion of reagents and colorimeter tubes. Provided the standard and the unknown solutions were subjected to the same conditions it was thought that the results might be as accurate. To test this, the constant temperature bath was used in one set of determinations and in the other an ordinary ether can filled with water in which a piece of ice was constantly kept. The latter requires no watching and the color develops less rapidly, making it possible to read a solution containing three milligrams of citral without difficulty. At 15° the color developed by three milligrams is a little too intense. Results at 15° are slightly higher.

In preparation of aldehyde-free alcohol, it was found that three grams of metaphenylene diamin hydrochlorate per liter \* \* \* was sufficient provided the alcohol was boiled for eight hours and allowed to stand over night. \* \* \*

To ascertain to what extent the aldehyde in commercial spirits used for making up extracts might affect the citral determinations, a sample of ordinary 95 per cent alcohol was run in the same manner as an extract. It showed aldehyde equivalent to 0.031 gram citral. Results obtained with extracts may thus be a little higher than the truth for this reason.

*Linwood A. Brown:* In the determination of citral by the fuchsin method, the greatest objection to it is in obtaining alcohol perfectly free from aldehydes. The Dunlap method failed to give a perfectly aldehyde-free alcohol even after three times on the same samples of alcohol, i. e., the alcohol was subjected to the method three successive times.

The metaphenylene diamin hydrochlorid method gives the best results for this determination.

*W. A. Syme:* Commenting on the method for lemon extracts, I would say that the method for purifying the alcohol (with metaphenylene diamin) did not yield an alcohol that would not produce a color with fuchsin solution on two trials. This lessens the accuracy of the work. I would suggest that other methods of preparing alcohol be studied and that other solvents be tried.

A glance at the table is sufficient to show that the results obtained in 1907 were practically of no value. The discordant figures on sample No. 2 are in part explained by the fact that this extract was made up in 85 per cent alcohol and it was found later that globules of oil had separated and were floating on the surface. This fact is noted in the comments of Mr. Sy, who analyzed the sample some time after it had been made up.

The only other explanation offered, is that the analysts were not familiar with the method. So far as is known Mr. Seeker is the only collaborator who had had any such

experience and his results on samples Nos. 2 and 3 are very close to the theoretical figures. The following results were obtained on samples Nos. 4 and 5:

*Analyses of vanilla extract, 1907.*

Collaborator.	Sample No. 4.			Sample No. 5.
	Vanillin.	Coumarin.	Acetanilid.	Vanillin.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
J. M. Bartlett, Maine .....	0.122	0.079	0.056	<sup>a</sup> 0.133
A. L. Nehls, Illinois .....	<sup>b</sup> 0.244	.075	.045	.130
A. P. Sy, Washington, D. C. ....	.083	.076	.022	.....
T. F. Pappe, Philadelphia .....	.098	.080	.031	.103
J. C. Olsen, New York .....	.112	.066	.016	.145
L. A. Brown, North Dakota .....	.106	.082	.021	.112
Theoretical amount present .....	.0997	.0997	.0498	.....
Average .....	.104	.076	.032	.125
Greatest difference above amount present .....	.022	.....	.006	.....
Greatest difference below amount present .....	.014	.034	.004	.....
Difference between maximum and minimum .....	.039	.014	.040	.022

<sup>a</sup> Also reported 0.014 per cent coumarin in this sample.

<sup>b</sup> Omitted from averages.

COMMENTS OF THE ANALYSTS.

**A. L. Nehls:** It would be much more accurate to determine the specific gravity of the solution, and pipette off a known volume for analysis. It was found in this work that solution No. 4, standing in the beaker on the balance pan for five minutes lost 10 mg. This means that under ordinary conditions the third decimal place in the result is meaningless.

In this laboratory we use a new test for coumarin which was originated by Doctor Bryan. It depends on the fact that a drop of alcoholic potash solution containing 20 grams of potassium hydroxid to a liter will, when placed on crystals of coumarin, give a lemon yellow color. This color is very intense, but disappears rapidly. Neither vanillin nor acetanilid is affected by the reagent. The test is applied directly on the crystals by using a glass rod for a dropper, and the results have always been much more satisfactory than those obtained by any other test, because of its great delicacy. Both vanillin and coumarin give a yellow solution on standing for some time, but the crystals of vanillin remain uncolored until dissolved, while the coumarin crystals are intensely colored.

The color tests for acetanilid are not as satisfactory as they might be. In this work we found the polarizing microscope of the greatest use. There is little danger of confusing either of the three crystals which are likely to occur in a vanilla extract. The discrepancies in the results for vanillin are quite usual where the crystals are weighed directly from an ether solution. They seldom come out well, usually being colored, often nearly black. This can be remedied by another extraction, not with ether.

**A. P. Sy:** On testing this residue for acetanilid, (Ritsert's tests) no reaction for same could be obtained. As only 25 grams extract are taken, the residues actually obtained were 0.0029 gram and 0.0026 gram for the duplicates. Using 4 mg of pure acetanilid, no reaction could be obtained by Ritsert's tests as given in Bul. 107. Using chlorin water (U. S. P.) instead of a solution of chlorinated lime (1 : 200) a good reaction was obtained with 4 mg acetanilid; 2 mg gave fair test. The chlorin water is mixed with the acetanilid; a pink color forms in a few seconds, changing gradually to a purple and finally to a blue.

**J. C. Olsen:** *Vanillin:* The residue of the ether extraction for vanillin almost invariably contains a large amount of resin and other impurities. It has always been our custom to extract the vanillin with petroleum ether and deduct the residue from the weight of impure vanillin. It will be noted that the difference in results is considerable. In 18 determinations the impurity with the vanillin has varied from 2 to 30 mg, the average being 10.4 mg.

We have also found that an easier drying residue of vanillin has been obtained by extraction from the 2 per cent ammonia solution with chloroform.

*Coumarin:* For the separation of coumarin and acetanilid we have been unable to obtain petroleum ether with a boiling point 30-40° C. We have used gasoline, 86° B. On fractionating this naphtha, fractions boiling at 35-40, 40-45, 45-50, 50-60, etc.,

have been obtained. It has been our experience that the higher boiling fractions extract coumarin as well as vanillin fully as well as the lower boiling fractions.

*Acetanilid:* According to the official method this substance is to be looked for with the vanillin only when it has been found with the coumarin. In one of these three analyses of No. 4 reported all of the acetanilid was found with the vanillin.

The figures given by Mr. Olsen on vanillin by extraction with petroleum were as follows: No. 4, 0.080; No. 5, 0.054, from which it would appear that the extraction was not prolonged sufficiently.

*Linwood A. Brown:* Sample No. 5: The vanillin in this sample was somewhat impure owing to coloring matter from which I was unable to purify it.

The results would seem to show that as far as vanillin is concerned the method is satisfactory. The average on both vanillin and coumarin, however, indicates that some of the latter is weighed as vanillin. The coumarin figures are uniformly low, as are those for acetanilid, with one exception. One collaborator reports entire failure of the Ritsert's test for acetanilid as given in the provisional methods, and suggests a modification.

#### WORK OF 1908.

The work for 1908 was confined to the colorimetric method for the determination of citral in lemon extracts. Fifteen sets of samples were sent out to collaborators who had previously worked with the method, and reports have been received from twelve. As the method had been rather severely criticised by some of the members of the American Extract Manufacturers' Association, they were invited to name two collaborators, and selected Mr. Edward Kremers, of the Wisconsin State College, and Mr. Baer, of St. Louis. Samples were sent to both, and Mr. Kremers forwarded his set to I. W. Brandel, of the University of Washington. The following description of the method to be used was sent to each collaborator:

#### DETERMINATION OF CITRAL IN LEMON EXTRACT.

##### *Reagents.*

*Aldehyde-free alcohol.*—Allow alcohol (95 per cent by volume) containing 5 grams of metaphenylene diamine hydrochlorid per liter to stand for twenty-four hours with frequent shaking. (Note, nothing is gained by previous treatment with potassium hydroxid.) Heat under a reflux cooler for at least eight hours, longer if possible (often twenty-four hours are necessary), allow to stand over night and distil, rejecting the first 10 and last 5 per cent which come over. Store in a dark, cool place in well-filled bottles.

*Fuchsin solution.*—Dissolve one-half gram of fuchsin in 250 cc of water, add an aqueous solution of  $\text{SO}_2$  containing 16 grams of the gas and allow to stand until colorless, make up to one liter with distilled water. This solution should stand twelve hours before using and should be discarded after three days.

*Standard citral solution.*—One milligram of c. p. citral per cubic centimeter in 50 per cent by volume aldehyde-free alcohol.

##### *Apparatus.*

*A cooling bath.*—To be kept at from  $14^\circ\text{C}$ . to  $16^\circ\text{C}$ . The aldehyde-free alcohol, fuchsin solution, and comparison tubes are to be kept in this bath.

*Colorimeter.*—Any form of colorimeter using a large volume of solution and adapted to rapid manipulation may be used.

The comparison may also be made in Nessler or Hehner tubes.

##### *Manipulation.*

*Preliminary determination.*—Weigh in a stoppered weighing flask approximately 25 grams of extract, transfer to a 50 cc flask and make up to the mark at room temperature with aldehyde-free alcohol. Measure at room temperature and transfer to a comparison tube 2 cc of this solution, add 25 cc of the aldehyde-free alcohol (previously cooled in the bath) then 20 cc of the fuchsin solution (also cooled) and finally make up to the 50 cc mark with more aldehyde-free alcohol. Mix thoroughly, stopper, and place in the cooling bath for fifteen minutes. Prepare a standard for comparison at the same time and in the same manner using 2 cc of the standard citral solution. Remove and compare the colors developed. Calculate the amount of citral present and repeat

the determination using a quantity sufficient to give the sample approximately the strength of the standard. From this result calculate the amount of citral in the sample. If the comparisons are made in Nessler tubes, standards containing 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg should be prepared and the trial comparison made against these, the final comparison being made with standards between 1.5 and 2.5 mg varying but one-fourth of a milligram.

The following points are to be especially noted:

The aldehyde-free alcohol (25 cc) on standing for 20 minutes in the cooling bath with the fuchsin solution (20 cc) should develop only a faint pink coloration. If a stronger color is developed, treat again with metaphenylene-diamin hydrochlorid.

It is absolutely essential to keep the reagents and comparison tubes at the required temperature. Comparisons should be made within one minute after removing the tubes from the bath. Where the comparisons are made in the bath (this is possible only where the bath is glass) the standards should be discarded within twenty-five minutes after adding the fuchsin solution. Give samples and standards identical treatment.

Note on samples colored with turmeric whether or not the color interferes with the comparisons. On samples 2 and 3, after making determinations on the samples sent, repeat them, removing the colors as follows: After weighing the sample to be used for analysis in a glass stoppered weighing bottle, add a drop of concentrated hydrochloric acid and a small piece of fat-free woolen cloth, stopper and allow to stand over night. Remove the cloth washing with aldehyde-free alcohol and determine the citral in the colorless solution as usual. Repeat the above comparison heating the acidified sample and woolen cloth under a reflux cooler for a few minutes, cool, remove the cloth and determine the citral as usual.

The samples sent were as follows:

No. 1. A lemon extract containing 3,008 grams of 95 per cent alcohol and 192 grams lemon oil, the whole colored with turmeric.

No. 2. A terpeneless extract of lemon strengthened with citral; 300 grams lemon oil dissolved in 1,796 grams of 95 per cent alcohol; 2,070 grams of water were added and after standing over night the precipitated oil was removed and 3.76 grams of citral added. The whole colored with Naphthol Yellow S.

No. 3. A solution of citral in dilute alcohol (50 per cent volume) containing 3,000 grams alcohol, 3.6 grams c. p. citral making the actual percentage of citral 0.12 per cent. The whole colored with Naphthol Yellow S.

No. 4. A solution of citral in dilute alcohol (50 per cent by volume) containing 3,500 grams alcohol and 2.12 grams c. p. citral making the actual percentage of the latter 0.061. The whole colored with turmeric.

The results reported are given in the following table:

*Collaborative work on determining citral in lemon extracts, 1908.*

Collaborator.	Without removal of color.				After removal of color.			
					By heating.		At ordinary temperature.	
	No. 1.	No. 2.	No. 3.	No. 4.	No. 2.	No. 3.	No. 2.	No. 3.
R. W. Hiltz, Philadelphia, Pa.....	Per ct. 0.330	Per ct. 0.330	Per ct. 0.125	Per ct. 0.060	.....	.....	0.28	0.108
A. W. Hansen, New York.....	.322	a. 407	.136	.067	.....	0.110	.386	.136
W. L. Dubois, Buffalo, N. Y.....	.328	.329	.107	.056	0.140	.089	.233	.100
A. P. Sy, Buffalo, N. Y.....	.318	.317	.116	.056	.110	.090	.278	.102
A. L. Cook, San Francisco, Cal.....	.286	.315	.118	.054	.228	.085	.188	.079
L. A. Brown, Agricultural College, North Dakota.....	.288	a. 370	a. 190	a. 100	.....	.....	.....	.....
F. D. Merrill, Detroit, Mich.....	.354	.324	.133	.064	.202	.031	.333	.080
R. S. Hiltner, Denver, Colo.....	.326	.316	.137	.084	.215	.137	.311	.138
C. O. Dodge, Washington, D. C.....	.308	.312	.133	.060	.....	.....	.294	.116
S. H. Baer, St. Louis, Mo. a.....	a. 380	a. 387	a. 165	a. 140	.....	.....	.....	.....
A. E. Taylor, Savannah, Ga.....	.360	.340	.125	.070	.290	.110	.290	.110
I. W. Brandel, Seattle, Wash.....	a. 143	a. 385	.109	.050	.....	.....	.....	.....
Averages.....	.322	.323	.124	.062	.....	.....	.....	.....
Maximum above average.....	.038	.017	.013	.022	.....	.....	.....	.....
Maximum below average.....	.046	.011	.017	.017	.....	.....	.....	.....
Theoretical amount.....	.....	.....	.12	.061	.....	.....	.....	.....

a Excluded from averages.

*R. W. Hiltz:* The methods submitted for this work were adhered to with the exception that in the removal of color from samples 2 and 3 the portions were weighed out into the 50 cc graduated, glass-stoppered flask, acidified as directed, and the piece of fat-free woolen cloth added (about 1.5 inches square). After standing over night the volume was completed with aldehyde-free alcohol, *without* removing the cloth.

Preliminary tests of the samples were made against a series of standards, but all final determinations were made by matching in the colorimeter. Final comparisons were always arranged so that the depths of tints compared were within 10 per cent, generally less, of equal strength.

Results reported are calculated from averages of four to five readings made in rapid succession with columns of 40 mm and 30 mm, i. e., 8 to 10 readings. Comparisons on the different depths of liquid gave concordant results.

Color in samples 1 (turmeric) and 2 (Naphthol Yellow S) gave no trouble whatever in comparisons. The samples are so highly diluted in the final determination that the color does not interfere. On sample 3 (Naphthol Yellow S) considerably more of the original liquid is present in the comparison tube, due to its lower citral content, and a very slight modification of tint in depths of 40 mm was noticed. With depths of 30 mm there was no apparent difference and tints were matched with ease. Sample 4 (turmeric) behaved similarly to No. 3. In depths of 40 mm there was a slight difference of tint, because nearly 3.5 cc of the original liquid was present in the tube. This slight difficulty disappeared in depths of 30 mm. Samples 2 and 3 were very satisfactorily decolorized by the treatment with the cloth. However, in so far as ease of comparison is concerned this treatment seems superfluous if comparisons are made with comparatively short columns of liquid, as above noted.

*A. W. Hansen:* The operator could not see that the color interfered with the comparisons.

*W. L. Dubois:* The comparisons were made in wide Nessler tubes graduated to 100 cc which were cooled to 15° in a large bath and for comparison placed in a tall beaker containing water at 15° and around which was wrapped a piece of white paper, the beaker being set on a white surface and lifted therefrom a few inches at the time of reading. The color in samples 2 and 4 did not seem to interfere with the determinations. The fuchsin sulphite solution when made as directed retained a slightly brownish tint. The fuchsin, however, which we had available for the preparation of this solution was not labeled c. p. and this possibly may have accounted for our failure to get a perfectly colorless solution.

*C. L. Cook:* None of the readings of any of the samples was interfered with by the presence of the coloring matter used. It was found necessary to allow the fuchsin solution to stand at least forty hours before a blank could be obtained with the aldehyde-free alcohol we were able to distil.

*F. D. Merrill:* Samples 1 and 4 colored with turmeric gave a color differing somewhat from the standard used in the determination of citral. In Nos. 2 and 3 colored with Naphthol Yellow S less difficulty was experienced in matching colors with the standards in the determination of citral when the original extract was used, but when the sample was decolorized by either method suggested it had a very different color as compared with the standard used in citral determination, and great difficulty was experienced in matching colors.

*R. S. Hiltner:* The small amount of turmeric in samples No. 1 and No. 4 did not interfere perceptibly with the color comparisons.

Sample No. 2, when heated with hydrochloric acid and woolen cloth under reflux condenser as directed, turned brown, apparently due to decomposition of citral. A somewhat similar change took place with No. 3, but to a less degree.

The same result was obtained on these two samples by simply acidifying with hydrochloric acid and treating at once with fuchsin reagent as by allowing the acidified solution to stand over night in contact with wool.

I was unable to secure alcohol that would not respond to the fuchsin test for aldehyde, even after prolonged standing and heating with m-phenylene diamine hydrochlorid.

Besides the figures obtained by the trial method, Mr. Hiltner, of the Denver Food Inspection Laboratory, submitted a set obtained by a method devised by himself using metaphenylene diamine as a substitute for the fuchsin sulphite reagent. The writer makes the following claims for the method:

First. Since there is no color reaction with acetaldehyde, more correct results may be secured in the analysis of commercial extracts.



In the preparation of these extracts, ordinary rectified alcohol is, of course, used. Such alcohol always contains more or less acetaldehyde. Any general reagent for aldehydes, like fuchsin, therefore tends to give too high results for citral because of the reaction on the acetaldehyde present.

Second. It is unnecessary, as stated, to use especially purified alcohol free from aldehydes.

Third. All the operations may be carried on at room temperature.

The following figures were submitted on the official samples: No. 1, 0.251; No. 2, 0.305; No. 3, 0.117; No. 4, 0.061.

Nos. 1 and 2 are somewhat below the average figures submitted by the collaborators. Nos. 3 and 4 are much closer to the actual amount present than those obtained by Mr. Hiltner with the method under trial. As the method was called to the referee's attention only a few days before the meeting, no opportunity was offered to test it this year.

#### GENERAL DISCUSSION OF RESULTS.

The results obtained on the official samples as a whole exceed greatly the expectations of the referee.

When twelve different analysts are working even with a well-established method under varying conditions, experience has shown that some discordant results are apt to be obtained. When like discrepancies have been obtained with the official methods for nitrogen and potash, it would seem that the results, in the present case, are highly satisfactory.

It appears to be of no advantage to remove the color before making the determinations; in fact, several of the collaborators are of the opinion that it renders the solutions harder to read. The work done at Washington also indicated that there was little advantage to be obtained, certainly not sufficient to offset the loss of citral. The results were slightly better on the alcoholic solutions of citral than upon the extracts. They were better on the terpeneless extract than on the extract containing lemon oil. This is, in all probability, due to the effect of the non-aldehydic constituents upon the color of the fuchsin solution. Where the colors are not of like tint, considerable experience is required in order to correctly match them.

On the final comparisons the standard and sample must contain approximately equal amounts of citral; a deviation of over 10 per cent is not allowable.

The method is not difficult of manipulation, but does require pure reagents, especially in the case of aldehyde-free alcohol. It is highly probable that the greater part of the discordant results are due to the latter. Given a cologne spirit of good quality, there seems, however, to be no reason why good results should not be obtained. It is recommended that the method as submitted for the determination of citral in lemon extracts be adopted provisionally by the association.

#### REPORT ON SPICES.

By A. L. WINTON, *Associate Referee.*

The attention of the associate referee was directed to the adulteration of paprika with olive oil, and the methods of detecting this form of adulteration, by papers presented by Doolittle and Ogden and by Loewenstein at the New Haven meeting of the American Chemical Society. Although the time was short for giving this matter suitable attention, a circular letter was sent out on September 5 to such chemists as had previously expressed a willingness to cooperate, and later, samples of two kinds of paprika were distributed, one purporting to be pure, the other adulterated with olive oil.

The methods submitted for study are as follows:

### METHODS.

#### NON-VOLATILE ETHER EXTRACT.

Dry in a desiccator over night or until the moisture is largely removed a sufficient amount of the material to yield an extract of from 0.2 to 0.25 grams. Extract according to the official method for the determination of crude fat (Bul. 107, Rev., p. 39, 5 (b) (1)), collecting the ether solution in a tared flask. Dry the extract at 100° C. for 15-minute periods until constant weight is secured.

#### IODIN NUMBER.

Determine by the Hanus method (Bul. 107, Rev., pp. 136-7), using the extract obtained as described in the preceding section.

Great care should be exercised in weighing the flask, both before and after extraction; as an error of 1 milligram is equivalent to an error of over 0.5 in the iodine number. A glass-stoppered 200 cc Erlenmeyer flask may be used for the extraction and also, without transfer, for the determination of the iodine number, although in our experience more accurate results may be secured by using a vial-mouth unstoppered flask of about 40 cc capacity, thus reducing the exposed surface to a minimum. In the latter case the flask, after dissolving the extract in chloroform and adding the Hanus solution, is introduced into a saltmouth, glass-stoppered bottle, broken with a glass rod and the titration carried out in this bottle in the usual manner.

It was suggested that in extracting the fat 3 grams of the pure paprika and 2 grams of the paprika adulterated with oil be used, thus securing amounts of extract suitable for determination of iodine number.

#### ALCOHOL EXTRACT.

Follow the official method (Bul. 107, Rev., p. 163).

#### DISCUSSION OF RESULTS.

The results obtained by the five analysts who took part in the cooperative work are given in the following table:

#### *Analysis of pure paprika and samples mixed with olive oil.*

Collaborator.	Pure paprika.			Paprika with olive oil.		
	Alcoholic extract.	Non-volatile ether extract.	Iodine number.	Alcoholic extract.	Non-volatile ether extract.	Iodine number.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>	
Genevieve Imus, Minnesota.....	13.36		76.5	14.96		97.4
C. D. Woods, Maine.....	13.33		76.2	14.97		96.7
		5.43	82.0		15.30	76.1
		5.81	77.4		13.18	80.7
C. P. Wilson, Washington, D. C.....	10.44	6.10	105.9	13.43	13.76	106.4
C. S. Brinton, Philadelphia.....	12.8	5.27	105.8	13.4	12.01	
	12.7	5.36	105.2	13.35	12.10	115.05
C. I. Lott, Chicago.....	A 10.62	5.52	114.6	12.92	12.58	115.2
		5.54	112.3		12.64	108.0
		5.37	116.7		12.72	112.0
	B 10.50	5.45	117.2		12.71	116.1
		5.47	112.9	12.76	12.52	107.2
		5.32	117.7		12.42	115.6
	C 10.75	5.47	115.2		12.51	111.4
		5.46	113.1	12.96	12.80	105.1
		5.40	115.1		12.75	105.8
					12.79	116.1
					12.60	117.6

α Average.

*Genevieve Imus:* This collaborator states that through a misunderstanding the portions taken for analysis were weighed out after drying the materials in a desiccator. For this reason the percentages of alcohol extract and nonvolatile ether extract are not comparable with those given by the other analysts and are not given in the table.

*C. D. Woods:* The ether extract in the determinations made by the method described not being complete, other trials were made, using different quantities of the material and extracting for longer periods. The results are given in the following table:

*Analyses of pure paprika and samples adulterated with olive oil, varying weight of sample, and time of extraction (Woods).*

PURE PAPRIKA.

Weight used.	Time of extraction.	Non-volatile ether extract.	Iodin number of non-volatile ether extract.
Grams.	Hours.	Per cent.	
3	16	5.43	82.0
3	16	5.81	77.4
3	24	5.48	87.3
3	24	5.74	79.3
3	24	5.91	77.9
1	100	13.15	-----
1	100	13.93	42.6

PAPRIKA ADULTERATED WITH OLIVE OIL

2	16	15.30	76.1
2	16	13.18	80.7
1	24	12.23	93.5
1	24	13.65	84.0
1	24	13.85	84.1
1	100	18.37	66.2
1	100	18.70	65.8

Mr. Woods comments on the above results as follows:

I note that Doolittle and Ogden report a much higher iodine number than any of my results would indicate and also that their results are very concordant. With directions as given I fail to see how one could place any reliance on the results of this determination. It may be possible that by running the ether extraction for an exact definite time results can be obtained agreeing reasonably close with each other, but I doubt this somewhat, for it has been our experience that some determinations extract much faster than others, depending on the rate of flow of the ether and the type of extractor used, and that until the extraction is complete there is no surety that two determinations will agree at any given time during the process.

These few determinations seem to indicate that if the iodine number is made on the complete ether extract other material besides fat (resins, etc.) will so increase the weight that the value of the iodine number will be reduced, while, if the determination is made before the extraction is complete, the results can not be depended on to agree.

C. S. Brinton comments on his results as follows:

The iodine numbers on the nonvolatile ether of the samples prepared with oil did not agree, and I am reporting only the average of results obtained. I was very much surprised to find the iodine number of the nonvolatile ether extract in the pure sample so much lower by the method you suggest than that obtained by the method used by Doolittle, but this is easily accounted for, because a long extraction with ether carries other material which is not readily soluble in ether and would not be found in the ether extract when a shorter extraction time is used. From the results obtained by this method I do not think that it would be advisable to use an official ether extract for the determination of the iodine number, as by so doing we are liable to overlook samples prepared with olive oil, the presence of which would be revealed by using Doolittle's method.

C. P. Wilson stated that he was not entirely satisfied with the results because with the apparatus he used he found it necessary to dissolve the fat before removing it from the flask in which it was recovered by the extraction.

*C. I. Lott:* In order to secure evidence with regard to the accuracy of the sampling, analyses were made of three bottles (A, B, and C) of each paprika. The discrepancies in the determination of the iodine number were attributed partly to differences in the amount of extract obtained occasioned by the removal of different amounts of the difficultly soluble resins and partly to errors in the process of determining the iodine number. It was suggested that possibly in the earlier determinations the extract was not completely dissolved in the chloroform preliminary to the Hanus solution. In the later determinations special effort was made to secure a complete solution. With this precaution the following results were obtained: Pure paprika, 116.7, 117.7, 115.1; paprika with olive oil, 116.1, 116.1, 117.6. Further experiments are needed to ascertain whether or not a better agreement of results can be secured by observing special precautions in dissolving the extract.

#### CONCLUSIONS.

The radical difference in the results reported by the different analysts in the determination of nonvolatile ether extract and the iodine number of the extract may be in part explained by differences in the extraction apparatus employed and in the rate of extraction, some of the analysts securing an extract which contained a much greater amount of resins than that obtained by the others, which resins have a much lower iodine number than the fatty oil. This explanation, however, does not account for many of the differences. For example, Messrs. Woods and Lott obtained practically the same percentages of nonvolatile ether extract in the pure paprika, but one reports an average iodine number of about 80 and the other of about 115. On the other hand, Mr. Wilson obtained the highest percentage of nonvolatile ether extract, and Mr. Brinton the lowest, yet both secured practically the same results on the iodine number.

The results reported indicate either that the method of securing the nonvolatile ether extract for the determination of iodine number is seriously at fault, or else special precautions, yet to be determined, are necessary to the success of the process. The results are not only widely discrepant, but they fail to throw any light whatever on the question of adulteration.

#### RECOMMENDATIONS.

It is suggested that during the ensuing year the following methods be studied: First, extraction on filter paper, with ether, as followed by Doolittle and Ogden,<sup>a</sup> and, second, shaking for a definite time with a definite volume of ether and evaporation of a portion of the filtered extract. It is believed that satisfactory results can be obtained only by a purely conventional method, using the same weight of material, the same volume of ether, and the same time of extraction. It may be found important, however, to use such portions of the ether solutions as will yield in all cases approximately the same amount of nonvolatile ether extract. The results obtained in the determination of alcohol extract throw no light on the question of added oil.

#### REPORT ON COLORS.

By H. M. LOOMIS, *Associate Referee.*

The work of the past year has been chiefly on the identification of colors. For this purpose twelve samples of colored food products were prepared in the laboratory, using the purest colors available, and samples of each were sent to six cooperating chemists. It is only just to state that many of the colors used were not furnished as food colors by the manufacturers. In this work the endeavor has been to prove that the colors used were simple commercial colors, and not mixtures, without special regard to the presence of mineral salts, etc.

<sup>a</sup>J. Amer. Chem. Soc. 1908, 30: 1481.

Since the promulgation of F. I. D. 76 of the Board of Food and Drug Inspection, allowing the use of certain coal-tar colors in food products and prohibiting all others, it has become quite necessary for food chemists to make a study of the methods of identifying colors to find out with what degree of accuracy these methods serve their purpose. In making this study it is of course very essential to work with pure colors, and as the time available for this work would not allow of preparing these colors in the laboratory, there were used colors furnished by manufacturers, who in most cases gave both the commercial and the scientific name of these samples, and upon them such tests were made as seemed necessary to establish fully the fact that they corresponded with the names given and were simple unmixed colors.

No originality in the methods of testing is claimed, the standard works of reference on the subject having been freely consulted. In every case the well-known tests by color reactions in aqueous solution, on the dyed fiber, and with concentrated sulphuric acid on the dry color were used. This includes a test for mixed colors made by sprinkling dry color on a surface wet with water or concentrated sulphuric acid. In addition the following tests were made on the separate colors:

#### TARTRAZIN:

*Precipitation by alcohol:* Concentrated aqueous solution + 95 per cent alcohol = crystalline yellow precipitate.

*0.1 per cent aqueous solution + stannous chlorid* = yellow precipitate, soluble in oxalic acid solution (10 per cent).

*0.1 per cent aqueous solution + barium chlorid solution* = yellow precipitate.

*0.1 per cent aqueous solution + calcium chlorid solution* = no precipitate.

*Conclusion:* Pure color, S. & J. 94.

#### SAFFRON:

*Test for coal-tar colors; microscopic examination.*

*Conclusion:* Pure color.

#### NAPHTHOL YELLOW S:

*Color reaction with stannous chlorid and ammonia.* Test for organic sulphur.

*Deflagration test:* Heated on platinum foil. Takes fire explosively.

*Solubility in ether:* Nearly insoluble in neutral or acid solution.

*0.1 per cent aqueous solution + barium chlorid solution (10 per cent)* = orange precipitate, insoluble in acetic acid.

*0.1 per cent aqueous solution + calcium chlorid solution (10 per cent)* = no precipitate.

*0.1 aqueous solution + lead acetate* = orange precipitate, soluble in acetic acid.

*0.1 per cent aqueous solution + cobalt chlorid and caustic soda* = olive-green precipitate.

*Remarks:* Color nearly pure. Contains small amount of unsulphonated naphthol yellow.

#### TROPAEOLIN O O:

*Reduction of color by stannous chlorid in acid solution; separation of para aminodiphenylamin from alkaline solution by ether.* Melting point, 61° to 62° C.

*Precipitation by salt:* 0.1 per cent solution of color + few drops 10 per cent sodium chlorid solution = precipitate of color.

*0.1 per cent aqueous solution + barium chlorid (10 per cent)* = colored precipitate, like  $\text{Fe}(\text{OH})_3$ .

*0.1 per cent aqueous solution + calcium chlorid (10 per cent)* = colored precipitate, like  $\text{Fe}(\text{OH})_3$ .

*Conclusion:* Pure color, S. & J. 88.

#### ERYTHROSIN: (Color used in sample 3 C.)

*Aqueous solution, pink fluorescent (shows presence of other colors besides erythrosin.)*

*Color extracted from acidified aqueous solution by ether.* Ether solution washed several times, evaporated and color dried.

*Halogens:* Chlorin, bromin, and iodine found in color qualitatively. (Mulliken "Identification of Organic Compounds," p. 13). Determination of bromin and iodine by Janasch and Aschoff method gave 17 per cent bromin and 9.7 per cent iodine.

*Color a mixture of eosin colors containing chlorin, bromin, and iodine.*

#### RHODAMIN:

*Contains no bromin or iodine; 0.4 per cent ash; insoluble, even on boiling in caustic potash solution; sp. gr. 1.3.*

*Aqueous solution pink; yellow fluorescence, which disappears on warming and reappears on cooling.*

0.01 per cent aqueous solution + stannous chlorid solution = bright crimson precipitate, purplish by transmitted light.

0.01 per cent aqueous solution + tannin reagent; test for basic color = precipitate.

Benedikt's test with zinc and ammonia: (Allen, "Commercial Organic Analysis," vol. 3, part 1, page 322.)

Conclusion: Pure color, S. & J. 504.

#### ROSE BENGAL:

Qualitative analysis shows halogens, iodine, and chlorine—no bromine.

Benedikt's test with zinc dust and ammonia. (See Allen, loc. cit.)

Benedikt's test: Boiling with caustic potash solution. (Sp. gr. 1.3.) (See Allen.)

Color separated from acidified aqueous solution as in the case of erythrosin.

Quantitative determination of iodine and chlorine.

Chlorine determined by silver nitrate after removal of iodine by nitrous acid and carbon bisulphid.

Iodine determined from total halogens by difference.

Chlorine, 8.89 per cent; iodine, 49.5 per cent; ratio = 1 to 5.56.

Ratio of halogens in tetraiodo-dichloro-fluorescein = 1 to 7.2.

Ratio of halogens in tetraiodo-tetrachloro-fluorescein = 1 to 3.6.

Conclusion: Color a mixture of the two Rose Bengals, S. & J. 520 and 523.

#### PHLOXIN:

Qualitative analysis shows presence of halogens, bromine, and chlorine; no iodine.

Determination of bromine by Mohr's method gave 39.8 per cent in color, purified by extraction with ether.

Chlorine, from total halogens by difference = 11.9 per cent.

Ratio — chlorine : bromine = 1 : 3.34.

Benedikt's test with zinc dust and ammonia. (See Allen.)

Benedikt's test with boiling potassium hydroxid solution. (See Allen.)

Conclusion: This color is a mixture of the two phloxins, S. & J. 518 and 521.

#### COCHINEAL RED A, S. & J. 106:

Tested for mixed color by precipitating part of color from solution with salt, filtering and dyeing wool to same depth with filtrate and solution of precipitated color. Both dyeings were nearly the same shade, indicating fairly pure color.

Dry color sprinkled on concentrated sulphuric acid shows small amount of foreign color.

Reduction with stannous chlorid and hydrochloric acid, making alkaline with sodium hydroxid and extracting, gave very little ether-soluble matter. This shows absence of colors yielding ether-soluble bases on reduction.

Conclusion: Color is fairly pure, but contains a small amount of foreign color.

#### FAST RED C, S. & J. 103:

Tested for mixed color, as in the case of cochineal red A, by fractional precipitation with salt and by sprinkling on concentrated sulphuric acid. Small amount of foreign color shown.

Color is fairly pure, but contains a small amount of foreign color.

#### PONCEAU 2R or 3R:

0.1 per cent aqueous solution + barium chlorid solution (10 per cent) = crimson precipitate, insoluble in acetic acid.

0.1 per cent aqueous solution + calcium chlorid solution (10 per cent) = no precipitate.

0.1 per cent aqueous solution + lead acetate solution (10 per cent) = crimson precipitate.

Color reduced with stannous chlorid and hydrochloric acid. Solution made alkaline with caustic soda and distilled with steam. Liquid amido compound distils over, which could not be solidified in ice water. Boiling point about 215° C. This shows the amido compound to be xylydin.

Conclusion: Color is ponceau 2R or xylydin red, S. & J. 55.

#### ACID GREEN:

Solubility in absolute and 95 per cent alcohol; no sign of mixed color by sprinkling on wet filter; no chlorine in the ash.

Conclusion: Pure color, S. & J. 435.

#### PERSIAN BERRY EXTRACT:

Reactions correspond very closely to those of a buckthorn berry extract prepared in the laboratory.

The accompanying table shows the results obtained in the identification of the colors. Considering the fact that three of the collaborators had never undertaken work on the identification of colors before, the results appear to be quite satisfactory with regard to the coal-tar colors.

No.	Material colored.	Color used.	Reports of analysts.				
			C. B. Brinton.	Hare, Mitchell, and Pringle.	F. O. Woodruff.	Hayward and Allen.	
1 F	Lemon extract.....	Tartrazin (Bad.) S. & J. 94.	Tartrazin. S. & J. 94.	Wood yellow T. (Tartrazin.)	Tartrazin.....	Not reported.....	E. J. Shanley.
2 F	Lemon extract.....	Saffron	Saffron.....	Chrysoidin	Vegetable color	do.....	Saffron
3 F	Lemon extract.....	Naphthol yellow S (Yellow Y. M.), S. & J. 4.	Naphthol yellow S, S. & J. 4.	Yellow Y. M. (R. H.) (Naphthol yellow S.)	Naphthol yellow S.....	do.....	Naphthol yellow. S. & J. 4.
4 F	Lemon extract.....	Tropaeolin O. O. (Cassella) S. & J. 88.	Tropaeolin O. O. (Possibly metanil yellow) Not reported.....	Orange IV. (Tropaeolin O. O.) S. & J. 88.	Metanil yellow (S. & J. 95.)	do.....	Orange IV. (Tropaeolin O. O.) S. & J. 88.
1 C	Cherries.....	Cochineal. Red. A. (Bad.) S. & J. 106.	Not reported.....	Cochineal Red A.....	Ponceau 6R. S. & J. 108.	do.....	Scarlet 6R. S. & J. 108.
3 C	Cherries.....	Mixture.	Eosin A. S. & J. 512.	Fosin A. S. & J. 512.	Fosin.....	do.....	Eosin. S. & J. 512.
4 C	Cherries.....	Rose Bengal (Bad.) S. & J. 520-523.	Rose Bengal. S. & J. 520.	Rose Bengal.....	(?)	do.....	Rose Bengal.
V	Pumpkin pulp.....	Ponceau 2R (Sch.) S. & J. 55.	Ponceau 3R. S. & J. 56.	Ponceau 3R. S. & J. 56.	Xyldidin Red. Ponceau 2R. S. & J. 55.	do.....	Cochineal Red A. S. & J. 106.
VI	Sugar sirup.....	Buckthorn (Persian berry extract).	Similar to Weld.....	Alizarin yellow A?.....	Caramel.....	Not reported.....	Fustic.
VIII	Sugar sirup with caramel.	Phloxin (Bad.) S. & J. 518 & 521.	Phloxin. S. & J. 521.	Phloxin.....	Phloxin.....	do.....	Eosin A. S. & J. 512.
IX	Sugar sirup with caramel.	Fast Red C (Bad.) S. & J. 103.	Not reported.....	Bismarck brown.....	Fast red C.....	do.....	Fast Red D. (S. & J.) (107.)
X	Sugar sirup.....	Acid green (Cassella.) S. & J. 435.	Light green S. F. yellowish. (Acid green.) S. & J. 435.	Acid green O. O. (Sch.) S. & J. 435.	Acid green.....	Acid green.....	Acid green. S. & J. 435.

## NOTES AND COMMENTS BY THE COLLABORATORS.

C. S. Brinton used the tables of Rota and others given by Allen,<sup>a</sup> Schultz and Julius, "Organic coloring matters," and Circulars 25 and 35, Bureau of Chemistry. Considerable difficulty was encountered in some cases in isolating color from fruit pulp and sirup. Double-dyeing method was used for extracting color from material, and color was obtained in aqueous solution by extracting wool with ammonia. Sample VI gave considerable trouble, and definite report was not made.

F. O. Woodruff used chiefly tables of Green, Yeoman, and Jones,<sup>b</sup> also tables in Allen and in Schultz and Julius, and Circular 35, Bureau of Chemistry. He says: "Three difficulties attending identification are: (1) A commercial dye from different manufacturers varies in purity and therefore in properties, though bearing the same or a synonymous trade name; (2) amount of color on dyed fiber or in color solutions affects the nature of the reactions therewith; (3) ordinary description of color reactions varies with the observer and does not allow of fine distinctions."

Hare, Mitchell, and Pringle used Rota's table and those in Circular 35, Bureau of Chemistry. They comment as follows: "We find Rota's scheme quite valuable in assisting us in the general classification of the dye. An accurate and complete color chart would be a great aid, especially to those not used to making sharp color distinctions."

E. J. Shanley used the tables in Allen and Circular 35, Bureau of Chemistry.

## RECOMMENDATIONS.

It is recommended—

(1) That an effort be made to obtain authentic samples of vegetable or natural coloring matters, such as are used in food products. This work should be assigned to such men as are in a position to obtain authentic samples, for it is well-nigh impossible for one person to obtain any considerable number of such samples and to ascertain their source and method of preparation;

(2) That characteristics of vegetable coloring matters and methods for identification be studied;

(3) That synthetic preparations of pure colors for standards be made;

(4) That the separation and identification of mixed colors be studied.

The president announced the following appointments as members of Committee A on recommendations of referees: R. J. Davidson, J. P. Street, J. G. Lipman, B. L. Hartwell, and W. A. Withers.

The association adjourned until 2 o'clock.

## THURSDAY—AFTERNOON SESSION.

## REPORT ON MEAT AND FISH.

By F. C. WEBER, *Associate Referee*.

In view of the fact that no work has ever been reported to the association on this subject, it seemed to the referee that some results showing the degree of accuracy of some of the chemical methods ordinarily employed in separating protein nitrogen, and at what point they show deterioration of meats, might be of interest. Owing to the nature of the work and the difficulty of keeping samples uniform, no attempt was made to secure collaborative work.

## SAMPLES.

The determinations here reported were made on three samples of chicken meat. Six young market chickens were obtained, killed, dressed, and allowed to stand in the ice box over night. The next morning the flesh was separated from the bones

<sup>a</sup> Commercial Organic Analysis, vol. 3, part 1.

<sup>b</sup> Soc. Dyers and Colorists, 1905, 21: 236.



and skin and thoroughly ground and mixed by passing six times through a meat chopper. It was then divided into two equal portions, one marked "fresh" and the other, after the addition of 0.1 per cent of boric acid, was marked "preserved."

The third sample represents the meat from three cold-storage drawn chickens, in storage twenty-six months, treated in the same manner as above, but without the addition of boric acid, and marked "stored." Each sample was placed in a screw-cap Mason jar and allowed to stand for one week, at laboratory temperature during the day, and in an ice box at night. During this time samples were taken for analysis on the first, second, third, sixth, and seventh days of standing. Every precaution was observed to guard against loss of moisture during the removal of the sample, as a result of which the moisture content remained very constant.

#### METHODS.

The following determinations were made at each of the periods cited: Moisture, total nitrogen, ammonia nitrogen, and, in the aqueous extract at room temperature and with ice water, nitrogen was determined as total, coagulable, amido, and ammonia. The difference between the sum of the coagulable and amido nitrogen and the total soluble nitrogen is considered as proteoses and peptones. The fat was determined once at the beginning of the experiment.

*Moisture* was determined on a 2-gram sample, dried in a water oven for ten hours. The loss of weight was calculated as moisture.

*Fat*.—The dried sample from the moisture determination was ground with dry sand and extracted with anhydrous ether in a Knorr extractor for twenty-four hours for the determination of fat.

*Nitrogen* determinations were made in the Nitrogen Section of the Bureau of Chemistry by Mr. H. W. Houghton, using the Gunning modification of the Kjeldahl method.

The *ammonia nitrogen* was determined on from 5 to 10 grams of sample distilled from a 750 cc flask, after the addition of 250–300 cc water and 10 grams magnesium oxid. The distillate was collected in standard acid and the ammonia nitrogen determined after a one-half hour distilling, 150 cc being distilled off. The distillation was continued for three half-hour periods, 150 cc of water being returned to the flesh between each distillation. The results reported represent the sum of the three half-hour periods.

*Water-soluble nitrogen [at room temperature (23°–25° C.) and with ice water (8° C.)]*: Twenty grams of the well-mixed sample of meat were weighed into a 450 cc Erlenmeyer flask, 250 cc of water added, and shaken for three hours in a shaking machine. In the case of ice-water extract chopped ice was added from time to time, the volume in the flask being kept constant by decanting the excess of water into a second flask. After being shaken the required length of time, the flasks were placed in the refrigerator over night, a small quantity of thymol and phenol having been added as a preservative. The next day they were poured through linen bags and extracted with room temperature and ice water, respectively, by vigorous manipulation with the hands and successive portions of water, till the final extract gave a negative biuret reaction. The extraction was very tedious and required, at first, an entire day for completion, using from 2,200 to 2,500 cc of room-temperature water, and from 1,800 to 2,000 cc of ice water. The room-temperature extract was made up to a volume of 2,500 cc, while the ice-water extract was made up to 2,000 cc throughout the experiment, though the latter extractions, particularly on the last two days, were completed with from 1,400 to 1,800 cc water. After making to volume and thoroughly mixing, the solutions were filtered through 6-inch funnels containing a 38.5 cm S. & S. 588 folded filter paper. The first 750 cc which ran through was discarded (in the case of the room-temperature extract this was used for the ammonia determination); the second quantity, 600 cc to 800 cc, was used for the water-soluble nitrogen determinations.

The filtration of the solutions of the first three extractions was very simple, the solutions running through the paper readily, though the second portion was still somewhat cloudy. As the samples spoiled, the extraction became more easy and the filtration more difficult, until on the last two days it was quite difficult to obtain sufficient solution to make the determinations. This filtered extract was entirely clear. The total nitrogen in the aqueous extract was made on 100 cc of the solution.

*Ammonia nitrogen* was determined on 500 cc of the room temperature extract, by distillation with magnesium oxid.

The coagulable protein nitrogen was determined in a sample of 200 cc of the water extract. This was placed in a 300 cc evaporating dish and evaporated on the steam bath to a volume of 40 cc. The solution was neutralized with tenth-normal sodium hydroxid, using phenolphthalein as indicator, then replaced on the steam bath and allowed to evaporate for ten minutes, filtered on a plain filter, and washed with hot water. The filter and precipitate were transferred to a Kjeldahl flask and the nitrogen determined.

*Amido nitrogen:* The coagulable protein filtrate was made up to 100 cc volume and 50 cc employed for the amido nitrogen determination. The 50 cc were placed in a 100 cc graduated flask, 15 grams of sodium chlorid added, and the flask well shaken and placed in an ice box. A 24 per cent solution of tannin was prepared, filtered, and placed in the ice box. After one hour 30 cc of the 24 per cent tannin solution were added to each flask and the two flasks filled to the mark with ice cold water. The flasks were thoroughly shaken and stood in the ice box over night. A blank must be carried out simultaneously, as the best tannin contains some nitrogen. The solutions are filtered into 50 cc flasks and the nitrogen determined in the 50 cc. The nitrogen figure thus obtained multiplied by two, minus the nitrogen of the blank, gives the amido nitrogen in 50 cc of the coagulable filtrate.

The sum of the amido and coagulable nitrogen subtracted from the total soluble nitrogen is considered as proteoses and peptones. No effort was made to separate the albumoses, proteoses, and peptones. All the results are calculated to a moisture and fat-free basis and are also expressed in per cent of the total nitrogen of each day's analysis.

The ice water extractions were made by Mr. H. I. Amoss and the coagulable and amido nitrogen separations by Mr. F. C. Cook, both of the Bureau of Chemistry.

The methods as selected, while not representing all that might have been employed, were those that have been generally used in the Bureau of Chemistry, and it is hoped that the work may be used as a starting point in this subject and serve to show the accuracy of the methods when applied to meats in a progressive state of deterioration.

#### DISCUSSION OF RESULTS.

The moisture results show very little change throughout the period, the average in the case of the fresh and preserved samples being 73.00 and 71.70 per cent for the storage sample. There was 4.12 per cent of fat in the fresh chicken and 4.09 per cent in the storage. The results on total nitrogen (see table, page 48) are as uniform throughout as the nature of the material and the accuracy of sampling would permit, and serve to show that there is no gaseous loss of nitrogen, while the ammonia nitrogen (that determined directly on the sample, as well as that determined in the extract) is markedly increased throughout and very uniform, particularly in the case of the stored and preserved samples. The amount is quite small at the time of the first analysis and remains so till the third analysis (made after standing two days), when the storage sample contains a little more than the other samples. From this point the increase is rapid. The variations in percentage amounts are from practically 1 per cent in all cases on the first analysis, to 11, 15, and 13 per cent for the fresh stored and preserved samples, respectively, on the last analysis, after seven days standing. The ammonia results on the water extract were unfortunately not made on the first day. They show practically the same results as those determined directly, but are not quite so uniform and not so high in amount. In the case of the formation of ammonia, the increased amount seems to begin to be formed after two days standing.

In connection with these changes it may be well to state here the changes in the samples which could be observed macroscopically. At the time of first analysis the samples were fresh, the storage sample showing a characteristic dried appearance. After standing one day they were practically the same, though what may be termed a slight fermenting action seemed to be taking place. On standing two days the samples had begun to deteriorate, especially the fresh and stored sample, while the preserved sample appeared fairly fresh. After three days standing, the deterioration was more

marked. A slight odor of spoiled meat was noticeable, more markedly in the fresh and stored meat than in the preserved. After standing six days the odor was quite bad; the samples had lost their texture and there was no doubt that they had spoiled. No difference in their physical condition could be detected after standing seven days that was not noticeable after six days standing.

The nitrogen determined in the water-soluble material at room temperature shows the total nitrogen extracted to be largely increased during the experiment, the first decided increase showing in the samples after standing two days. The coagulable nitrogen shows but a slight tendency to increase, the most marked and uniform change being in the stored sample. The amido nitrogen is not very uniform and shows a tendency to decrease especially where the samples are in an advanced stage of putrefaction. The nitrogen here termed proteoses and peptones is markedly increased during the final days of the experiment, the storage sample again showing a more uniform change. The increase of ammonia nitrogen in the water extract conforms to that determined directly, but is not quite so large in amount.

The nitrogen in the ice water extract in the various forms separated shows the same general trend as does that of the room temperature extract, though the amounts extracted are usually not so large.

The graphic charts, figs. 3 and 4, show these changes more plainly. It is quite noticeable throughout that the results on the storage sample are very uniform and progressive and, moreover, in all but two instances, in all the determinations, the results on the first analysis show the storage sample to be lower in the various constituents than the fresh samples. The same general tendency seems to run throughout the experiment though one would expect the storage meat to deteriorate more rapidly.

Taking into consideration the variations in the determinations and the limitations of the methods themselves, there does not appear to be a very clearly defined point at which deterioration can be said to begin, unless it is shown by the ammonia and water-soluble total nitrogen determinations. The increase in these constituents coincides with the macroscopical observation and physical appearance of the sample. The use of ice water in the extraction is unnecessary, as the methods employed are not of sufficient accuracy to detect the greater changes from day to day in the early stages, much less any change which may be due to enzymic action during the process of extraction.

It seems probable from the results that the determination of ammonia may be a valuable asset in showing the first indications of changes, as these results are the most uniform and progressive. A large amount of work has recently been done on the methods for the determination of ammonia in animal and vegetable materials. Richardson <sup>a</sup> after considerable experimenting on the ammonia nitrogen determination, and in which he extracted the meat with 60 per cent alcohol and distilled with magnesium oxids, aspirating air through the flask, and distilling under reduced pressure, finally adopted the method as outlined above as best suited to the purpose.

His results on pure ammonium chlorid distilled in a vacuum with magnesium oxid and 60 per cent alcohol are nearly theoretical. This is in substance the method as now employed in the determination of ammonia in urine and might be adapted to this work.

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<sup>a</sup> J. Amer. Chem. Soc., 1908, 30: 1515.

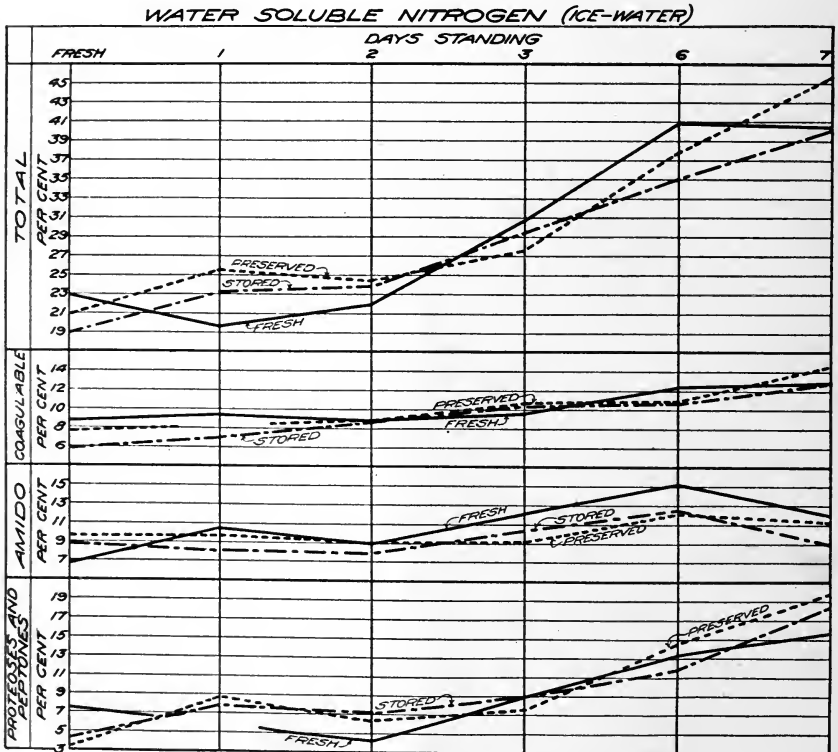
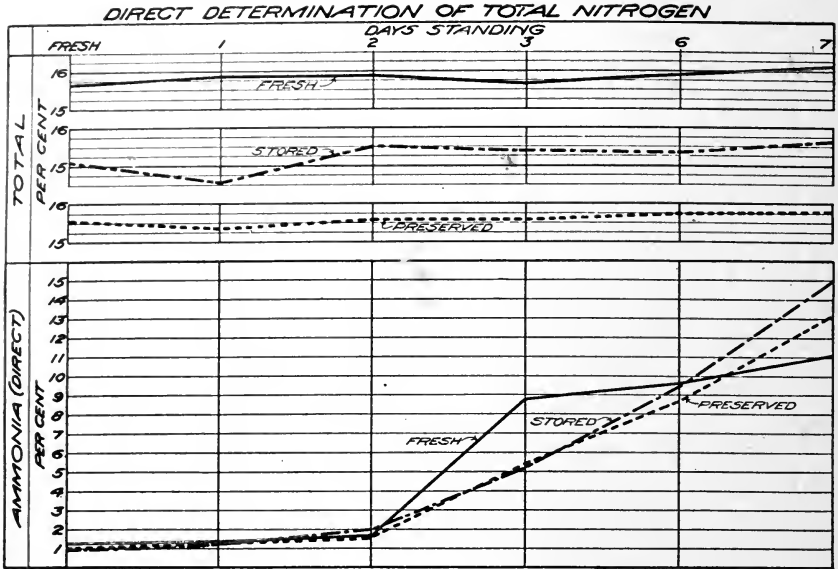


FIG. 3.—Direct determinations of total nitrogen and ammonia and changes in the nitrogenous constituents (soluble in ice water) of fresh, cold-stored, and preserved chicken meat, during seven days.

## WATER SOLUBLE NITROGEN (ROOM TEMPERATURE)

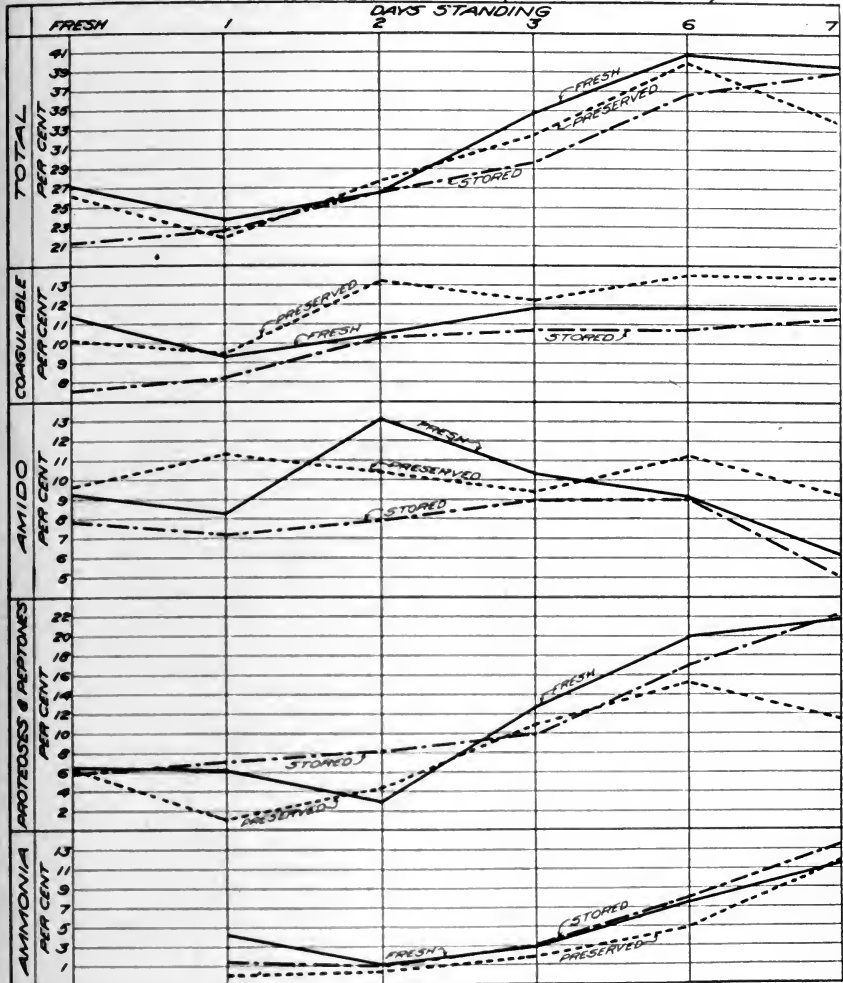


FIG. 4.—Changes taking place during seven days in the nitrogenous constituents (water-soluble at room temperature) of fresh, cold-stored, and preserved chicken meat.

*Nitrogenous bodies in water extracts of fresh, stored, and preserved chicken meat.*

[Water-free and fat-free basis. Black figures are results expressed in percentage of total nitrogen.]

Date.	Days old.	Description.	Total nitrogen.			Ammonia nitrogen.			Nitrogen in water extract (room temperature) 22°-23° C., 35--		
			Fresh.	Stored.	Preserved.	Fresh.	Stored.	Preserved.	Fresh.	Stored.	Preserved.
1908.	0	Fresh; stored sample had characteristic dried appearance.....	Per cent. 15.63	Per cent. 15.11	Per cent. 15.57	Per cent. 0.198	Per cent. 0.142	Per cent. 0.153	Per cent. 4.27	Per cent. 3.23	Per cent. 4.09
Oct. 13	1	All samples comparatively same; no signs of deterioration.....	15.86	14.56	15.35	1.27	.94	.98	27.32	21.38	26.27
Oct. 14	2	Samples beginning to deteriorate; more marked in fresh and stored; preserved, fairly fresh.	15.90	15.51	15.63	1.33	1.18	1.26	3.76	3.29	3.36
Oct. 15	3	Slight odor noticeable, more marked in fresh and stored; samples were spoiling and becoming soft.	15.66	15.42	15.62	1.67	1.97	1.52	23.71	22.60	21.89
Oct. 16	6	Odor quite bad and samples spoiled; were soft and "mushy".....	15.93	15.37	15.75	8.81	5.19	5.40	4.21	4.11	27.77
Oct. 19	7	Same as on 19th.....	16.15	15.66	15.83	1.38	1.46	1.37	5.44	4.56	5.06
Oct. 20						9.60	9.49	8.70	31.71	29.57	32.39
						11.21	15.00	2.09	40.80	36.69	39.87
								13.20	39.69	39.02	33.92

Total soluble.

## Nitrogen in water extract (room temperature) 22°-23° C., as—

Date.	Days old.	Description.	Coagulable.			Amido.			Proteoses and peptones.			Ammonia.		
			Fresh.	Stored.	Pre-served.	Fresh.	Stored.	Pre-served.	Fresh.	Stored.	Pre-served.	Fresh.	Stored.	Pre-served.
1908.	0		<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	
Oct. 13	0	Fresh; stored sample had characteristic dried appearance....	1.79	1.15	1.59	1.46	1.20	1.51	1.02	0.88	0.98	0.66	0.20	
Oct. 13	1	All samples comparatively same; no signs of deterioration....	11.45	7.61	10.21	9.34	7.94	9.69	6.53	5.82	6.29	4.16	1.37	
Oct. 14	1	All samples comparatively same; no signs of deterioration....	1.48	1.21	1.45	1.31	1.05	1.74	0.96	1.03	1.17	0.66	0.20	
Oct. 15	2	Samples beginning to deteriorate; more marked in fresh and stored; preserved, fairly fresh.	9.33	8.31	9.45	8.26	7.21	11.34	6.05	7.07	1.11	4.16	1.37	
Oct. 16	3	Slight odor noticeable, more marked in fresh and stored; samples were spoiling and becoming soft.	10.50	10.25	13.18	13.08	7.93	10.36	2.83	8.25	4.29	1.01	0.77	
Oct. 19	6	Odor quite bad and samples spoiled; were soft and "mushy"....	11.81	10.70	12.23	10.28	8.95	9.35	12.64	9.86	10.82	2.94	3.05	
Oct. 20	7	Same as on 19th.....	11.80	10.74	13.52	9.17	8.98	11.24	19.84	16.98	15.11	7.53	8.00	
			1.91	1.82	2.12	0.97	0.78	1.45	3.49	3.51	1.80	1.82	2.10	
			11.83	11.62	13.39	6.13	4.98	9.16	21.61	22.41	11.37	11.27	13.41	

Nitrogen in water extract (tee water) 8° C., 48—

*Nitrogenous bodies in water extracts of fresh, stored, and preserved chicken meat—Continued.*

Date.	Days old.	Description.	Total soluble.			Coagulable.			Amido.			Proteoses and peptones.		
			Fresh.	Stored.	Pre-served.	Fresh.	Stored.	Pre-served.	Fresh.	Stored.	Pre-served.	Fresh.	Stored.	Pre-served.
1908.			<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
Oct. 13	0	Fresh; stored sample had characteristic dried appearance....	3.60	2.87	3.23	1.37	0.89	1.19	1.04	1.31	1.51	1.19	0.67	0.53
Oct. 14	1	All samples comparatively same; no signs of deterioration...	<b>23.03</b>	<b>18.99</b>	<b>20.75</b>	<b>8.77</b>	<b>5.89</b>	<b>7.64</b>	<b>6.65</b>	<b>8.67</b>	<b>9.69</b>	<b>7.61</b>	<b>4.13</b>	<b>3.40</b>
Oct. 15	2	Samples beginning to deteriorate; more marked in fresh and stored; preserved, fairly fresh.	3.12	3.37	3.91	1.50	1.03	2.26	1.07	1.19	1.51	1.15	1.15	1.35
Oct. 16	3	Slight odor noticeable, more marked in fresh and stored; samples were spoiling and becoming soft.	<b>19.67</b>	<b>23.15</b>	<b>25.47</b>	<b>9.16</b>	<b>7.07</b>	<b>14.72</b>	<b>10.53</b>	<b>8.17</b>	<b>9.84</b>	.....	<b>7.89</b>	<b>8.79</b>
Oct. 19	6	Odor quite bad and samples spoiled; were soft and "mushy"...	3.49	3.71	3.81	1.40	1.35	1.39	1.42	1.22	1.42	.....	.....	.....
Oct. 20	7	Same as on 19th.....	<b>21.95</b>	<b>23.92</b>	<b>21.38</b>	<b>8.81</b>	<b>8.70</b>	<b>8.89</b>	<b>8.93</b>	<b>7.87</b>	<b>9.09</b>	<b>4.21</b>	<b>7.29</b>	<b>6.39</b>
			4.82	4.53	4.29	1.51	1.59	1.68	1.90	1.58	1.42	1.40	1.37	1.19
			<b>30.78</b>	<b>29.38</b>	<b>27.16</b>	<b>9.61</b>	<b>10.31</b>	<b>10.76</b>	<b>12.13</b>	<b>10.25</b>	<b>9.09</b>	<b>8.91</b>	<b>8.88</b>	<b>7.62</b>
			6.51	5.39	5.96	1.97	1.63	1.73	2.42	3.92	1.91	2.12	1.84	2.32
			<b>40.87</b>	<b>35.07</b>	<b>37.84</b>	<b>12.36</b>	<b>10.60</b>	<b>10.98</b>	<b>19.19</b>	<b>12.19</b>	<b>12.13</b>	<b>13.31</b>	<b>11.97</b>	<b>14.73</b>
			6.54	6.28	7.22	2.09	2.01	2.31	1.92	1.39	1.78	2.54	2.88	3.13
			<b>40.49</b>	<b>40.10</b>	<b>45.60</b>	<b>12.94</b>	<b>12.84</b>	<b>14.59</b>	<b>11.89</b>	<b>8.88</b>	<b>11.51</b>	<b>15.73</b>	<b>18.39</b>	<b>19.77</b>



## REPORT ON THE ADULTERATION OF DAIRY PRODUCTS.

By HERMANN C. LYTGOE, *Associate Referee.*

The referee, with the help of Messrs. Nurenberg and Marsh, assistant analysts of the Massachusetts State board of health, has made a study of the different methods for the preparation of milk serum and for the detection of calcium succinate in cream. As a result of this work it is apparent that the provisional method for the preparation of milk serum needs no modification, but the method of Baier and Neumann for the detection of sucrose in milk or cream should be made provisional. The work done is embodied in the two following articles.

### A COMPARISON OF METHODS FOR THE PREPARATION OF MILK SERUM.

The samples of milk used in this investigation were all milked in the presence of an inspector or an analyst of the Massachusetts State board of health and represented nearly all breeds of dairy cattle, particularly the Holstein, Ayrshire, Dutch Belted, and grade Holstein cows. The methods employed were the provisional (acetic acid) method, natural souring,<sup>a</sup> calcium chlorid method,<sup>b</sup> and asaprol method.<sup>c</sup> The details of the methods other than the provisional methods are as follows:

*Natural souring method.*—Allow the samples to sour spontaneously and filter off the serum.

*Calcium chlorid method.*—Place 90 cc of milk in a flask, add 0.75 cc of calcium chlorid solution—sp. gr. 1.1375 (when diluted 1:10 this solution reads 26 on the immersion refractometer at 17.6° C.), shake thoroughly, close the flask with a cork carrying a glass tube to act as a reflux condenser, place in a boiling water bath for twenty minutes, cool to 20°, mix the condensed water and serum without shaking, and filter.

*Asaprol method.*—The precipitating solution is made by dissolving 30 grams of asaprol and 55.8 grams of crystallized citric acid in 1 liter of water. If the refraction of this solution is not 36.3 on the scale of the immersion refractometer at 20°, add water or citric acid to make it so. Mix equal volumes of the above solution and the milk, shake well, and filter.

In the accompanying table are the results of the refraction of the milk serum prepared from milk samples of known purity when two or more methods were applied to the same sample of milk. The asaprol method is by far the easiest of manipulation. It gives the clearest serum in the least time and shows the lowest refraction with the least variation. Unfortunately pure asaprol is very difficult to obtain, and, owing to the fact that it decomposes readily, it is not an easy matter to prepare different solutions that will give identical sera with the same sample of milk. The calcium chlorid method is the most difficult of manipulation and is liable to give a cloudy serum rather troublesome to read, but the results are lower than those obtained by the acetic acid method and not so variable. The natural souring method is too slow for ordinary use, but is valuable in the hot weather if the milk is nearly sour when it reaches the analyst. Four years' experience with the provisional method has shown it to be reliable, easy of manipulation, and to give concordant results.

<sup>a</sup> Matthes and Müller, Zts. öffentl. Chem., 1903, 10: 173.

<sup>b</sup> Ackerman, Zts. Untersuch. Nahr. Genussm., 1907, 13: 186.

<sup>c</sup> Baier and Neumann, Zts. Untersuch. Nahr. Genussm., 1907, 13: 369.

*Refraction of milk sera from known purity milk of individual cows.*

Acetic acid.	Method.			Acetic acid.	Method.		
	Natural souring.	Calcium chlorid.	Asaprol.		Natural souring.	Calcium chlorid.	Asaprol.
46.2	47.7			43.0	43.2		
45.9	44.6		38.4	43.0	42.8		
45.8	44.0			43.0	42.3		
45.7	43.4	40.1	37.0	43.0	42.0		
45.6	43.5			43.0	41.7		
45.5	41.5	38.7	37.1	43.0	41.5		
45.1	41.5	39.0	36.8	43.0	41.4	39.1	36.4
44.9	41.5	39.8	36.1	43.0	41.1	38.4	36.6
44.8	43.8			43.0	40.9		
44.8	43.7	39.2	36.0	42.9	43.6		36.5
44.7	43.5		36.6	42.9	43.0		
44.6		38.5	36.6	42.9	41.4	39.0	36.7
44.5	45.0			42.8			37.4
44.8	42.8		35.7	42.7		38.8	
44.4	43.8		36.7	42.7		38.0	
44.3	43.0			42.6	41.3	38.1	36.1
44.3	42.8			42.5	42.2	39.3	36.3
44.3	42.3	39.0	37.0	42.5	41.5		
44.2	41.2	38.6	37.0	42.5		38.2	36.6
44.2	41.0			42.4	43.3		36.4
44.1	43.0	39.1	36.7	42.3	43.7	39.0	37.0
44.1	40.7	38.2	36.8	42.3	41.9		36.8
44.0	42.2	38.9		42.3	41.6	39.8	36.6
43.9	44.5			42.3	40.8	38.8	36.7
43.9	42.6			42.2	42.0		37.0
43.8	44.0			42.2	41.0		
43.8	44.0			42.1	44.0		36.8
43.8	43.0		36.7	42.1	43.7		36.6
43.8	41.6		37.5	42.0	41.0		
43.7	44.2		37.5	42.0	40.3	37.1	36.2
43.7	42.6			42.0	40.2	36.8	35.8
43.7	42.4			41.8	40.5		
43.7	41.5		36.9	41.7	40.9	38.2	36.1
43.6	43.0			41.7		35.0	
43.6	43.0	40.0	36.3	41.7	40.0	36.8	36.2
43.6	42.0	38.6	36.3	41.6	43.9		36.0
43.5	43.5			41.5	40.4	36.4	35.7
43.5	42.8			41.4	40.3	38.4	35.6
43.5	41.0	38.4	36.3	41.3	40.3		
43.4	43.1			41.2	40.0		
43.2	43.3			40.6	40.7	36.6	35.8
43.2	42.2			40.5	39.3		
43.2	41.8	38.9	36.6	40.4	38.3		
43.2	40.9		37.4	40.0	40.1		
43.0	43.7						

*Mixed milk of known purity.*

43.6	42.9	39.0	37.5	42.5	41.0	39.4	36.3
43.5	42.0	38.7		42.1	39.3		
43.4	40.8	38.2	36.7				

THE DETECTION OF CALCIUM SUCRATE IN MILK OR CREAM.

The calcium succrate used in this investigation was prepared by adding 2.5 parts, by weight, of sugar to 1 part of quick lime slaked in 8 parts of water, allowing to settle, and decanting the supernatant liquid. The sample polarized at 17.3° V. in the 200 mm tube and its alkalinity was 1.86 normal.

Leffmann's method<sup>a</sup> for the detection of calcium succrate in cream, using sesame oil and hydrochloric acid as the reagents, was found to be satisfactory only in the presence of larger quantities than are necessary to thicken cream, therefore it was abandoned.

The method of Baier and Neumann<sup>b</sup> was found to be entirely satisfactory for the detection of sugar, and is as follows:

<sup>a</sup> Chem. Ztg., 1906, 30: 638.

<sup>b</sup> Zts. Nahr. Genussm., 1908, 16: 51.

To 25 cc of milk or cream add 10 cc of a 5 per cent solution of uranium acetate, shake, allow to stand for five minutes and filter. If the filtrate is not clear pour it through the filter again. To 10 cc of the clear filtrate (in the case of cream use the total filtrate if less than 10 cc) add 2 cc of a cold saturated solution of ammonium molybdate and 8 cc of dilute hydrochloric acid (1 part of 25 per cent hydrochloric acid and 7 parts of water) shake well and place in a water bath at 80° C. for five minutes. If the sample is pure the solution will resemble a nickel sulphate solution, but if sugar is present it will be of a Prussian blue color. These different colors can be readily distinguished but it is advisable to compare with a standard blue solution made by adding a few drops of potassium ferrocyanid and 5 drops of 10 per cent hydrochloric acid to a solution of 1 cc of 0.1 per cent ferric chlorid in 20 cc of water.

**Alkalinity of ash.**—Evaporate 25 cc of cream to dryness, and burn to an ash in a muffle. Dissolve the ash in an excess of tenth-normal sulphuric acid, boil to expel the carbon dioxide and titrate back with tenth-normal sodium hydroxid, using phenolphthalein as the indicator. Express results as cubic centimeters of tenth-normal acid required to neutralize the ash of 100 grams of cream.

**Determination of calcium.**—Add acetic acid to the final solution from the above determination, heat to boiling, add 1 gram of sodium acetate and an excess of ammonium oxalate. Filter and wash the precipitated calcium oxalate with water, dissolve in hot dilute sulphuric acid, and titrate hot with tenth-normal potassium permanganate. The number of cubic centimeters of tenth-normal potassium permanganate multiplied by 0.0112 ( $4 \times 0.0028$ ) gives the percentage of calcium oxid in the sample.

The table appended shows the composition and reactions of pure and adulterated cream, using the Baier and Neumann method for calcium succrate. It is recommended that this method be distributed for criticism.

*Results on pure and adulterated creams using the Baier and Neumann method for calcium succrate.*

Pure cream.						Cream containing calcium succrate.					
Total solids.	Fat.	Ash.	Alkalinity of ash.	Calcium oxid.	Sucrose.	Calcium succrate added per liter.	Fat.	Ash.	Alkalinity of ash.	Calcium oxid.	Sucrose.
Per ct.	Per ct.	Per ct.	cc.	Per ct.		cc.	Per ct.	Per ct.	cc.	Per ct.	Present.
51.20	45.2	0.36	7.6	0.073	None.	5	37.9	0.50	19.2	0.147	Do.
49.98	43.2	.35	6.0	.073	None.	2	37.9	.46	13.2	.130	Do.
47.86	42.8	.39	6.4	.062	None.	5	41.4	.39	11.6	.095	Do.
47.12	41.4	.37	6.8	.069	None.	4	42.8	.43	10.0	.101	Do.
.....	41.4	.43	8.8	.099	None.						
.....	40.4	.38	7.6	.092	None.						
47.00	39.2	.40	8.0	.091	None.	.....	36.4	.39	16.0	.123	Do.
.....	39.2	.36	7.2	.085	None.	.....	39.8	.....	16.0	.143	Do.
45.02	39.6	.41	6.8	.083	None.	.....	28.8	.....	14.0	.135	Do.
43.08	36.8	.33	7.6	.094	None.	.....	39.8	.....	10.8	.130	Do.
42.80	37.2	.41	7.2	.086	None.	.....	35.6	.....	12.0	.141	Do.
42.75	36.8	.46	7.6	.083	None.						

## REPORT ON CEREAL PRODUCTS.

By E. F. LADD, *Associate Referee.*

During the past year considerable work was undertaken in our own laboratory upon cereal products, very little of which has as yet been completed. Therefore only a report of progress can be made. As the result of examinations made by A. S. Mitchell, chief of the St. Paul Food and Drug Inspection Laboratory, the following methods are suggested:

### METHODS FOR ANALYSIS OF CEREAL PRODUCTS.

#### MOISTURE.

Dry a convenient quantity of the flour (approximately 5 grams) at the temperature of boiling water in a current of dry hydrogen or in vacuo until it ceases to lose weight.

## ASH.

Char a convenient weight of the original sample (from 2 to 5 grams) in a platinum dish, in a muffle, at the lowest possible temperature until free from carbon. If carbon free ash can not be obtained owing to its fusibility, exhaust charred mass with water and proceed as under ash, Bulletin 107, page 38.

## CRUDE FAT (ETHER EXTRACT).

Extract a convenient quantity of the product (from 4 to 5 grams) as dried in the determination of moisture with anhydrous, alcohol-free ether, for 24 hours (with fine flour the addition of an equal weight of clean dry sand is frequently necessary). Dry the extract at the temperature of boiling water until it ceases to lose weight.

NOTE.—Iodin numbers should only be obtained upon the ether extract after purification by solution in petroleic ether, but are best made upon the petroleum ether extract.

## SOLUBLE CARBOHYDRATES (AS DEXTROSE).

Weigh 16 grams of flour into a 500 cc flask. Add 200 cc of water. Shake occasionally during one-half hour. Filter through a dry folded filter. To 50 cc of the filtrate add 5 cc of concentrated hydrochloric acid. Place the flask in water and invert at 70° C. for ten minutes. Cool, neutralize, and bring to 100 cc. Filter. Determine the reducing sugars with Fehling solution, by the official method, as described in Bulletin 107, calculating the reducing sugars as dextrose.

## CRUDE FIBER.

Determine the crude fiber in 2 grams of flour by the official method (Bul. 107), filtering through linen in a Büchner funnel.

## DETERMINATION OF MOIST GLUTEN.

Dough up 30 grams of flour with 18 cc of water conveniently in an 8-ounce mortar. Weigh off 16 grams of dough equivalent to 10 grams of flour. Place in water at room temperature for one hour and carefully wash out the starch over bolting cloth or a fine horsehair sieve. After expressing all globules of water, weigh the moist gluten upon a watch glass. Dry in a desiccator for 24 hours and complete drying in water oven.

## ACIDITY IN FLOUR.

Weigh 18 grams of flour into a 500 cc Erlenmeyer flask and add 200 cc of distilled water, previously freed from carbon dioxide by boiling in tin. Place the loosely stoppered flask in a water bath kept at 40° C. for 10 minutes, shaking repeatedly. Remove the flask and allow it to stand, with occasional shaking, at room temperature for one hour. Filter upon a dry folded filter, rejecting the first 10 cc and receiving the succeeding 100 cc in a graduated flask. Titrate the filtrate with twentieth-normal sodium hydroxid, using carefully neutralized phenolphthalein in alcohol as an indicator. Each cubic centimeter of twentieth-normal sodium hydroxid represents 0.05 per cent of acidity as lactic acid.

NOTE.—Results obtained with flour at temperatures of 15°, 20°, and 25°, respectively, indicate that the acidity in the solution increases with the temperature. The method outlined seems to give the maximum acidity.

## TOTAL NITROGEN IN FLOUR.

Determine the total nitrogen in 2 grams of flour according to the official method, preferably the Gunning method, Bulletin 107, page 7. The nitrogen times 6.25 gives total proteins.

## GLOBULIN AND ALBUMEN (EDESTIN AND LEUCOSIN) AND AMID NITROGEN.

Weigh 5 grams of flour into a 500 cc Erlenmeyer flask. Add 250 cc of sodium chlorid solution 1 per cent. Stopper and shake thoroughly. Let stand, with occasional shaking, for three hours. Filter on dry paper. Evaporate 100 cc of the filtrate to small volume in a Kjeldahl digestion flask with 5 cc of sulphuric acid. Add remainder of the

sulphuric acid and determine the nitrogen by the Gunning method. To a second 100 cc of the filtrate add 5 cc of phosphotungstic acid, 20 per cent solution; shake thoroughly, allow to settle, and filter by decantation. Wash slightly with water. Concentrate the filtrate with 5 cc of sulphuric acid in Kjeldahl flask and determine the nitrogen as amid.

Deduct the amid nitrogen from the nitrogen found in the first fraction to obtain the nitrogen as globulin and albumen. This figure times 6.25 gives globulin and albumen.

#### ALCOHOL SOLUBLE PROTEINS (GLIADIN).

Weigh 4 grams of flour into a 500 cc Erlenmeyer flask, add 200 cc of alcohol 0.90 sp. gr. Shake occasionally during three hours. Let stand 12 hours. Filter through a dried filter. Evaporate the alcohol from 100 cc of the filtrate after the addition of 5 cc of sulphuric acid and determine the nitrogen as alcohol soluble nitrogen. This figure, less the amid nitrogen, gives the alcohol soluble proteid nitrogen or gliadin.

#### GLUTENIN (DETERMINATION BY DIFFERENCE).

Deduct from the total nitrogen the salt soluble nitrogen plus the gliadin. This times 6.25 gives the glutenin.

#### GLIADIN BY POLARIZATION (METHOD OF SNYDER).

Weigh 15.97 grams of flour into a 300 cc flask. Add 100 cc of 0.90 sp. gr. alcohol. Shake at intervals during three hours and let stand overnight. Filter through a dry folded filter. Polarize in a 220 mm tube. Precipitate the proteids in 50 cc of the filtrate with 5 cc of Millon's reagent. Shake, filter, and polarize the filtrate in a 220 mm tube. Add 50 per cent to the reading and deduct the sum from the first reading. This difference times 0.2 gives the per cent of nitrogen as gliadin.

#### FAT DETERMINATION (BASSETT).

An effort was made to discover a method whereby the time for determining fat and moistures in cereal samples, especially flour, could be much shortened and without a sacrifice of accuracy. H. P. Bassett, of the North Dakota laboratory, was assigned some work along this line, the results of which are embodied in the following:

Fat in flour has been determined usually by the method given by Leach, which is outlined so as to be applicable to all food and feeding stuffs. However, in making fat determinations on flour by Leach's method considerable time is required, and unless special precautions are taken the analyst could never check himself. This, in any method, indicates inaccuracy. In examining the difficulties which might arise to affect this method, it was especially noted that oxidation might take place in drying the flour in a hot-water oven, as is generally practiced, since the fat in the flour is in a fine state of division, which gives the most favorable conditions for oxidation. Again, the special precaution of removing the last trace of moisture from the flour seemed to be an unnecessary point when the ether, as generally employed, contained probably ten times more water than was found in the dried flour.

The extraction by the Leach extractor is also slow, requiring sixteen hours, and in apparatus arranged in such a manner that it can not always be run with safety overnight. This means, then, three full working days before a determination can be made— one for the moisture determination and two for the fat determination.

In order to avoid these difficulties, the following method was developed:

Ten grams of flour were weighed into a tared gooch crucible, then placed in the ordinary gooch funnel, which was inserted into a rubber stopper in the top of a low bell-jar, which rested upon a ground-glass plate. Under the bell-jar and directly under the gooch funnel was placed a second glass plate to avoid the possibility of getting vaseline on the bulb, which was to catch the filtrate, vaseline being used to make an air-tight joint between the bell-jar and ground-glass plate. The gooch was now filled with ether six times, each time drawing off with the filter pump. The ether extract was collected in a bulb similar to those with a Soxhlet apparatus. This bulb was then removed and connected with a Liebig's condenser, and the ether distilled off with a 32 candle-power incandescent bulb, this being used as it avoided the possibility of the vapors of ether catching fire, and also has the additional advantage of not being so hot

as to easily burn the fat. The residue in the gooch crucible is now dried in an air oven and weighed, the loss in weight being equal to the fat and moisture. The fat having been determined, the moisture is easily obtained by difference.

The results by this method, however, are considerably higher than by the Leach method—often twice as much—but there is no difficulty in the analyst duplicating his results. Following are some of the figures obtained by this method:

*Comparison of methods for fat determinations.*

Number of experiment.	New method.		Leach method.	
	1.	2.	1.	2.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1.....	1.26	1.24	0.47	0.45
2.....	1.70	1.71	1.21	1.23
3.....	2.29	2.30	1.83	1.87
4.....	1.16	1.22	.68	.70
6.....	1.08	1.12	.65	.53
7.....	2.66	2.66	1.17	1.27
8.....	1.07	1.07	.60	.62
9.....	1.32	1.36	1.30	1.28
10.....	2.14	2.18	2.37	2.28
11.....	.97	.99	.70	.65
12.....	1.05	1.02	.65	.65
13.....	3.05	3.00	2.43	2.52

DISCUSSION OF RESULTS.

Comparing the results by the two methods, it is noticed at once that none of those by the new method checks those made by the old method, and it was thought at first that there might possibly be an error on account of the moisture present in the flour while extracting, the tests by the old method being carried out on dry flour. A large amount of moisture would probably cause some of the sugar-like substances to be extracted. In order to test this point, the following experiments were performed: Ten grams of flour were weighed out in a gooch crucible as before and placed in a large-mouthed bottle, which was closed with a two-hole rubber stopper. Carbon dioxide which had been dried over concentrated sulphuric acid was conducted through the bottle, the same being arranged in a water bath and heated for four days under these conditions. This was then extracted with ether, according to the new method. The results obtained checked exactly those extracted without drying.

*Results obtained by new method on dried and fresh flours.*

No.	Fat in dried flour.	Fat in fresh flour.
	<i>Per cent.</i>	<i>Per cent.</i>
1	1.21	1.24
2	1.22	1.26
3	1.24	1.23
4	1.24	1.22

The next point considered was to determine if flour dried in carbon dioxide, under these conditions, would give the same per cent of fat as by the new method or would check the old method. Therefore, 2 grams of flour were weighed out in a small test tube with a hole in the bottom, which was closed by an asbestos plug. This was then placed in the bottle described and dried for four days, after which it was extracted by the Leach method, the following results being obtained:

*Fat determinations, drying with carbon dioxid.*

New method.	CO <sub>2</sub> -dried flour by old method.
1.26	1.21
1.24	1.22
1.23	1.21
1.22	1.19

It is evident that when dried in carbon dioxid the flour does not undergo any oxidation, while from these results the point seems almost, if not completely, proven that it does undergo oxidation when dried in the open air, and for this reason it is hard to obtain results that check.

Numerous determinations have been made by this method and it has given perfect satisfaction as well as being extremely rapid. Time may be saved, as the gooch crucible does not need a new pad every time, the same one being used for at least fifteen determinations by simply knocking out the extracted flour when through. Further, the flask containing the fat may be used six or eight times without cleaning, where a number of determinations are being made.

This work was carried out with ether as the solvent, but chloroform, acetone, or benzin may also be used, similar results being obtained. The following results were obtained:

*Fat determinations by the new method, using different solvents.*

No.	Ether.	Chloroform.	Acetone.	Benzin.
1	1.24	1.26	1.29	1.20
2	1.26	1.27	1.26	1.21
3	1.23	1.25	1.27	1.23
4	1.22	1.29	1.28	1.22

These results become of value on account of the cost, chloroform and benzin being much cheaper than ether or acetone.

In some cases feed and foodstuffs are not well ground, nor capable of being ground as fine as flour. These of course would not extract by the above method readily, but may be extracted by means of the Soxhlet apparatus instead of the gooch crucible. The Soxhlet apparatus was used on flours and the results check those made with the gooch crucible very closely.

Either one of the solvents named may be used instead of ether, as a larger amount of solvent is required under such conditions, and unless special precautions are taken considerable loss may take place.

*Comparison of results on fat, using a gooch and the Soxhlet apparatus.*

Gooch crucible.	Soxhlet apparatus.
1.24	1.24
1.26	1.26
1.23	1.27
1.22	1.28

## MOISTURE DETERMINATION.

The moisture, as stated in the preceding method, may also be determined by drying the residue in a hot-water oven and then weighing the crucible and residue, the loss being equal to the weight of the fat and moisture from which the moisture may be determined. The following results were obtained and will be compared with the old method by Leach:

*Comparison of moisture determinations by two methods.*

New method.	Old method.	
<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
8.60	8.76	8.66
8.80	8.76	8.66
8.88	8.76	8.66
8.92	8.76	8.66

The results here, however, are not so close as in the fat determinations, but out of the numerous determinations that have been carried out in this laboratory the results have checked to within 0.2 to 0.3 per cent, and, in the majority of cases, within a hundredth of a per cent. The following table shows a few results which will give an idea of the accuracy of the method:

*Duplicate moisture determinations by new method showing degree of accuracy.*

Sample.	Per cent.	Per cent.
1	12.64	12.76
2	12.36	12.56
3	11.08	11.06
4	11.40	11.84
5	12.80	12.06
6	11.40	11.30
7	11.72	11.80

This method has been a means of saving much time, since a large number of such determinations were made during the flour investigation of the past year.

Considerable work was also undertaken in the study of the gluten and protein content of the flour, and a large number of methods were tested. Some results of special interest were secured, but owing to the illness of the assistant having this work in charge only progress in this direction can be reported.

## RECOMMENDATION.

It is recommended that for the coming year special attention be given to testing methods for the separation of the gluten constituents of flour, tests being made upon the several grades, as patent, first and second clears, and upon flours produced from different varieties and types of wheat.

## REPORT ON VEGETABLES (CANNED PEAS).

By W. L. DUBOIS, *Associate Referee.*

The work on this subject during the last year has been confined to the examination of canned peas for the purpose of distinguishing soaked peas from those canned when fresh. Such a distinction of course is made with quite a degree of certainty by a



simple examination of the physical appearance of the goods, noting especially the maturity and firmness of the peas and the consistency of the liquor. Soaked peas usually appear more or less broken and mashed and the most matured show well developed cotyledons and are packed in a liquor which is cloudy and starchy in appearance.

The maturity of the peas, however, can not be taken as conclusive evidence that the same have been soaked, because many well developed peas, very similar in appearance to those soaked before canning, are packed as numbers 4 and 5, Early June and Telephone peas, and are not soaked. Neither can the appearance of the liquor be finally relied upon, since the most mature, fresh peas are sometimes found in a liquor which is not clear and is more or less starchy; hence it is desirable to obtain data which would substantiate conclusions drawn from the physical appearance of the goods. To this end 73 miscellaneous samples of peas have been examined by the referee and on all these the weight of the liquor and drained substance, and the percentage of water in the drained substance were determined. These determinations alone are sufficient to distinguish the fresh peas and some of the more succulent grades from the soaked goods, the chief difficulty arising in differentiating between the soaked goods and the more matured peas put up in the fresh state. In the water content the latter did not differ very widely from the soaked peas. As will be seen from the table, the average content of water in 24 samples of soaked peas is 71.98 per cent, and the average of 18 samples of Early June and similar grades is 77.52 per cent. The highest moisture content of the soaked peas, however, exceeds that of the driest of the Early June peas, so that there is an overlapping of the results which makes it impossible to pronounce a conclusive opinion from these determinations alone.

More definite conclusions, however, may be drawn by also determining the crude starch. For this determination 15 grams of the ground drained material were hydrolyzed by hydrochloric acid according to the official method and all copper-reducing substance calculated as starch. The average of 16 results on soaked peas gave 14.45 per cent, the highest figure being 18.19 per cent and the lowest value 11.08 per cent, while the average starch content of 11 samples of matured peas canned in the fresh state was 10.87 per cent. Here again is an overlapping, the lowest results on the soaked peas, 11.08 per cent, being below the highest value obtained on the fresh grade, 14.38 per cent. This last sample, however, was probably misbranded, as will appear later. The average starch content on soaked peas, as far as determined, is approximately 4 per cent higher than that of Early Junes and those of similar quality. There is some difference, furthermore, in the specific gravity of the two grades, that of Early Junes running from 1.10 to 1.14, whereas the values obtained for soaked peas vary from 1.12 to 1.16. Taking all these figures into consideration, it seems possible by the determination of the water, starch, and specific gravity of the drained substance to obtain values which will supplement the conclusions drawn from the physical appearance of the goods.

The table gives in detail the results obtained. Samples numbered 81 and 82 are interesting in furnishing a test of the method suggested. These samples were labeled Early June peas, but both had the appearance of having been soaked. These were run at the same time as numbers 75 to 80, inclusive, and it will be seen how the starch content compares with the other samples labeled in the same way. By these values alone and the appearance of the goods it would be quite safe to conclude that they had been soaked. There is also a difference in the specific gravity, which is somewhat higher than in the other samples. This conclusion is further strengthened by the amount of water which is less than in the other samples.

The work seems to justify further investigation along the same line by the succeeding referee.

## Examination of canned peas to distinguish soaked goods.

## FRESH PEAS.

Number.	Grade and appearance.	Liquor.	Drained sub- stance.	Specific gravity.	Crude starch.	Protein.	Water.
		Grams.	Grams.		Per ct.	Per ct.	Per ct.
1	Large, firm, not succulent.....	194	398				78.61
2	First quality, sifted.....	201	389				82.68
3	Peas quite soft.....	193	400				81.87
4	Fairly soft.....	208	387				82.61
5	Soft, sifted, Telephone peas.....	196	396				82.94
6	Mealy, fancy, sifted, large peas.....	223	360				78.35
7	Fancy, sifted, sweet, good.....	187	378				86.84
8	do.....	197	390				78.53
9	do.....	222	365				78.87
10	Extra fancy, sweet, good.....	205	360				87.04
11	Extra fancy, good.....	190	380				86.87
12	Extra fancy, sweet, good.....	190	380				86.95
Surprise peas:							
13	No. 1 small.....	190	395				87.20
14	No. 2.....	220	383				86.57
15	No. 3.....	238	362				86.57
16	No. 4.....	202	400				82.52
17	No. 5.....	216	382				81.91
Alaska peas:							
18	No. 1.....	255	332				86.72
19	No. 2.....	245	344				82.37
20	No. 3.....	245	357				78.23
21	No. 4.....	220	387				76.34
22	No. 5.....	240	357				74.28
Admiral peas:							
23	No. 1.....	215	375				86.80
24	No. 2.....	203	387				84.59
25	No. 3.....	227	369				84.30
26	No. 4.....	220	369				80.83
27	No. 5.....	219	373				78.50
28	First quality; a few old.....	224	364				83.63
29	Good quality; a few old.....	209	381				79.29
30	Early June.....	205	380				79.59
31	Large, firm, about same as No. 30.....	197	356				86.73
32	Tom Thumb, very small, excellent.....	210	359				85.99
33	Petit Pois, small.....	238	334				85.99
34	Sifted Little Gem, small.....	233	353				85.91
65	Early June, good quality.....	215	383	1.12-1.16	13.88		70.79
66	Early June Extra No. 7, fair.....	265	305	1.10-1.14	12.13		74.55
67	Telephone, some broken and soft.....	218	374	1.10-1.14	12.15		74.62
68	Early June, a little mushy.....	232	372	1.10-1.16			75.10
69	Early June, appear soaked.....			1.10-1.14			
70	Early June, cloudy and starchy, firm.....			1.10-1.14			
71	Early June, cloudy and starchy, old.....			1.10-1.16			
72	Early June, poor quality, appear soaked.....			1.12-1.16			
73	Early June, good quality.....			1.08-1.16			
74	Early June, cloudy, poor, many broken.....			1.10-1.16			
75	Early June, firm, large, many hard.....	232	351	1.08-1.14	10.55		Lost.
76	Early June, medium size, good quality.....	229	366	Below 1.10	7.37		82.30
77	Early June, medium size, fair quality.....	250	335	1.08-1.16	12.00		75.45
78	do.....	Lost.	Lost.	Below 1.10	6.63		84.04
79	Early June, large, mealy, many hard.....	200	378	1.08-1.12	9.34		78.88
80	do.....	189	386	1.08-1.14	7.20		77.89
81	Early June, large, mealy, appear soaked.....	299	277	1.08-1.14	14.38		74.84
82	Early June, appear soaked.....	252	294	1.10-1.14	13.95		73.21

## Examination of canned peas to distinguish soaked goods—Continued.

## SOAKED PEAS.

Number.	Grade and appearance.	Liquor.	Drained substance.	Specific gravity.	Crude starch.	Protein.	Water.
		Grams.	Grams.		Per ct.	Per ct.	Per ct.
35	Soaked, some mushy	235	359	1.12-1.18	.....	.....	70.71
36	do.	255	335	1.12-1.16	14.05	.....	73.43
37	do.	243	324	1.12-1.16	.....	.....	71.67
38	do.	294	274	.....	.....	.....	74.36
39	do.	231	344	1.10-1.16	13.70	.....	62.07
40	do.	215	353	1.10-1.16	.....	.....	74.92
41	do.	187	405	1.12-1.16	.....	.....	73.68
42	do.	211	380	.....	.....	.....	70.91
51	Liquor very cloudy, soaked	274	306	.....	11.08	6.87	71.84
52	Soaked, very poor, mushy	222	280	.....	18.19	6.69	73.84
53	Soaked, liquor very cloudy	177	401	.....	14.23	6.56	72.46
54	Soaked, sample poor	152	418	.....	12.83	6.13	74.66
55	Soaked, cloudy, peas fair	213	368	.....	13.54	6.69	69.67
56	Soaked, cloudy, broken, and soft	175	408	.....	14.47	6.44	71.97
57	Soaked, mushy	187	390	1.12-1.16	13.06	6.06	74.10
58	Soaked, many broken	267	300	1.12-1.16	14.79	7.31	71.15
59	Soaked, very mushy	245	330	1.12-1.16	14.31	6.63	72.67
60	Soaked, fairly firm	253	341	1.12-1.16	14.77	6.19	73.05
61	Soaked, some soft, color fair	197	385	1.12-1.16	15.25	.....	71.00
62	Soaked, many poor	250	303	1.12-1.16	16.04	.....	71.70
63	Soaked, good, some black	214	323	1.12-1.16	15.77	.....	71.06
64	Soaked, somewhat mushy	304	258	1.12-1.16	14.34	.....	73.62

## REPORT ON THE SEPARATION OF MEAT PROTEIDS.

By P. F. TROWBRIDGE, Associate Referee.

During the past year no cooperative work has been asked for on the separation of meat proteids, but considerable work has been done at the Missouri experiment station, in connection with the general meat investigations, in the examination of cold water extracts of meat from composite samples of various wholesale cuts of steers of different ages and of different degrees of fatness.

In the preparation of cold water extracts the general plan as proposed by Grindley and Emmett<sup>a</sup> was followed with slight modifications.

With lean samples exactly 100 grams and with fat samples 150 grams were taken; in each case being distributed through eighteen beakers in about equal portions. The sample in each beaker was thoroughly mixed with 3 to 5 cc of cold neutral nitrogen-free distilled water and then with 50 cc of the same water. The mixture was allowed to stand about thirty minutes, with frequent agitation, and then poured through previously wetted filters. Each time the residue which was poured on the filter was returned to the beaker. To the residue in the beaker 25 cc of water were added and the meat residue thoroughly stirred and again filtered. This washing was continued till 225 cc of water were used; then the whole residue was transferred to the filter and washed twice with 10 to 15 cc of water each time.

The filtrates were combined, the flasks rinsed, and the total volume made up to 5,000 cc. This cold water extract was carefully mixed without undue agitation and filtered through a dry filter just before the aliquot portions were taken for analysis. (In all filtrations the funnel was made to touch the sides of the flask or beaker, so as to limit the amount of spontaneous coagulation.)

Triplicate samples were taken for analysis as follows:

*a, b, c.*—100 cc for total soluble nitrogen representing 2 grams of lean and 3 grams of fat sample.

*d, e, f.*—100 cc for total solids and ash representing 2 grams of lean and 3 grams of fat sample. \* \* \*

*m, n, o.*—200 cc for coagulable nitrogen representing 4 grams of lean and 6 grams of fat sample.

<sup>a</sup>J. Amer. Chem. Soc., 1905, 27: 661.

*p, q, r.*—200 cc for total albumose nitrogen; filtrate from *m, n, o*, representing 4 grams lean and 6 grams of fat sample.

*s, t, u.*—200 cc for total amido acid nitrogen representing 4 grams of lean and 6 grams of fat sample.

Samples *a, b, c* were transferred to 500 cc nitrogen flasks for the direct determination of nitrogen by the Kjeldahl-Gunning method.

Samples *d, e, f* were evaporated to dryness on the water bath, then dried to constant weight in air bath at 103°, and finally ashed at a dull red heat.

Samples *m, n, o* were treated with a slight excess of moist magnesium carbonate, *a* evaporated to about 30 cc, filtered and washed with hot water to which a little moist magnesium carbonate had been added. The precipitate and filter were transferred to 500 cc nitrogen flasks and the coagulum adhering to the sides of the beakers was removed with hot sulphuric acid and transferred to the corresponding flask. The nitrogen was determined in the usual manner.

The filtrates from *m, n, o, (p, q, r)* were concentrated to about 10 cc in small beakers and acidified with 1 cc of 50 per cent sulphuric acid, diluted to 30 cc and to each 50 grams of pure crystallized zinc sulphate were added. The mixture was then heated upon the water bath until the complete solution of the zinc sulphate took place. If too much zinc sulphate crystallized out upon cooling a little water was added, care being taken to have only a slight excess above saturation. The contents of the beakers were filtered through filters previously wet with a saturated solution of zinc sulphate slightly acidified with sulphuric acid. After the filtrate had completely drained through, the beaker and filter were washed three times with the saturated zinc sulphate solution, allowing the washing to drain completely before adding the next washing. The filter and precipitate were transferred to nitrogen flasks and each beaker washed with water and sulphuric acid, the washings being rinsed into the corresponding flask. If care has been used in avoiding an excess of zinc sulphate crystals there will be no trouble with bumping during the digestion for the determination of the albumose nitrogen.

Samples *s, t, u* were treated as for coagulable nitrogen. The filtrates from the coagulum, not to exceed 30 cc, were rinsed into 100 cc graduated flasks, 15 grams of sodium chlorid were added and dissolved by warming gently. The flasks were placed in the ice box until cooled to 15° C. A 24 per cent solution of tannic acid was made up, filtered, and cooled in the ice box. When both solutions were cooled, 30 cc of the tannic acid solution were added to each 100 cc flask, which was then filled to the mark with cold water; the contents of the flask were thoroughly mixed and allowed to remain in the ice box over night. The following morning they were filtered rapidly and 50 cc of the filtrate transferred to nitrogen flasks for the determination of the amido acid nitrogen. With all of the determinations blanks were made to correct for the nitrogen in the reagents. From these data the nitrogen present as peptone nitrogen was calculated.

The main purpose in the examination of the water extracts of the fresh meats has been to see if age of animal or condition of fatness has any influence upon the amount of water-soluble material, or upon its composition, also to what extent there is a variation in different parts of the animal. To this end the samples have been handled as nearly as possible in the same manner and with the same treatment after slaughtering. So far eight animals have been slaughtered and analyzed, but the data are still insufficient to admit of any general conclusions, and the present paper is to be regarded only as a report of progress. A tabulation of a few of the results is appended, selecting those for the round and rump, the rib and loin cuts of the first four animals slaughtered.

*a* Prepared by precipitating magnesium chlorid with sodium carbonate, heating, filtering, and washing until no chlorids remained in the filtrate.

*Distribution of nitrogen in cold-water extracts of wholesale cuts, free from bone, from four different steers.*

Sample.	Total solids.	Total nitrogen.	Coagu- lable nitrogen.	Albu- mose nitrogen.	Peptone nitrogen.	Amido- acid nitrogen.
<b>Steer No. 18:<sup>a</sup></b>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Round and rump.....	4.979	0.574	0.287	0.037	0.040	0.210
Rib.....	4.627	.540	.271	.039	.053	.177
Loin.....	4.778	.550	.263	.052	.064	.171
<b>Steer No. 121:<sup>b</sup></b>						
Round and rump.....	4.583	.528	.253	e.142	None.	.202
Rib.....	3.490	.413	.192	.010	.053	.158
Loin.....	3.531	.409	.195	.042	.010	.162
<b>Steer No. 505:<sup>c</sup></b>						
Round and rump.....	5.116	.587	.273	.035	.007	.272
Rib.....	4.117	.470	.199	.029	.021	.221
Loin.....	4.593	.534	.235	.041	.029	.229
<b>Steer No. 503:<sup>d</sup></b>						
Round and rump.....	5.389	.627	.294	.071	.036	.226
Rib.....	4.629	.530	.254	.033	.034	.209
Loin.....	4.348	.501	.234	.071	.015	.181

<sup>a</sup> Grade Shorthorn steer, 3 years old and extremely thin.

<sup>b</sup> Grade Shorthorn steer, 3 years old and moderately fat.

<sup>c</sup> Grade Hereford steer, 1 year old and moderately fat.

<sup>d</sup> Grade Hereford steer, 1 year old and in fair condition as a steer, much better condition than No. 18.

<sup>e</sup> The albumose precipitate was washed only once; results too high.

*Distribution of nitrogen in cold-water extracts of the lean of wholesale cuts (bone and fat hand separated) from three steers.<sup>a</sup>*

Sample.	Total solids.	Total nitrogen.	Coagu- lable nitrogen.	Albu- mose nitrogen.	Peptone nitrogen.	Amido- acid nitrogen.
<b>Steer No. 121:</b>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Round and rump.....	5.736	0.671	0.320	<sup>b</sup> 0.173	None.	0.258
Rib.....	4.548	.550	.251	.013	0.073	.213
Loin.....	5.398	.647	.306	.040	.041	.260
<b>Steer No. 505:</b>						
Round and rump.....	6.153	.710	.332	.039	.009	.330
Rib.....	4.939	.565	.240	.032	.025	.268
Loin.....	6.169	.719	.315	.054	.038	.312
<b>Steer No. 503:</b>						
Round and rump.....	5.980	.699	.330	.074	.040	.255
Rib.....	4.930	.567	.276	.028	.037	.226
Loin.....	5.510	.643	.301	.083	.023	.236

<sup>a</sup> Steer No. 18 excluded as it was the first one slaughtered, and the hand-separated fat was weighed separately but was ground with the lean for analysis.

<sup>b</sup> Albumose precipitate washed but once.

*Distribution of nitrogen in cold-water extracts of wholesale cuts, free from bone and fat,<sup>a</sup> of four different steers.*

Sample.	Total solids.	Total nitrogen.	Coagu- lable nitrogen.	Albu- mose nitrogen.	Peptone nitrogen.	Amido- acid nitrogen.
<b>Steer No. 18:</b>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Round and rump.....	5.740	0.662	0.331	0.043	0.046	0.242
Rib.....	5.645	.659	.331	.048	.064	.216
Loin.....	6.103	.703	.336	.066	.083	.218
<b>Steer No. 121:</b>						
Round and rump.....	6.072	.700	.335	<sup>b</sup> .188	None.	.268
Rib.....	5.612	.664	.309	.016	.085	.254
Loin.....	5.913	.685	.327	.070	.017	.271
<b>Steer No. 505:</b>						
Round and rump.....	6.631	.761	.354	.045	.009	.353
Rib.....	6.030	.688	.291	.042	.032	.323
Loin.....	6.705	.779	.343	.059	.043	.334
<b>Steer No. 503:</b>						
Round and rump.....	6.167	.717	.336	.081	.041	.259
Rib.....	5.453	.624	.299	.039	.040	.246
Loin.....	5.903	.680	.318	.096	.020	.246

<sup>a</sup> The weight of the actual amount of fat (ether-soluble) is deducted from the weight of the cut. This reduces all cuts to a fat-free basis.

<sup>b</sup> Albumose precipitate washed but once.

In the first table it will be noticed that if the data concerning the albumoses and peptones are omitted, the round and rump cuts in every case give higher figures than the other two cuts. When the hand-separated fat is eliminated we find less variation; the round and rump cut gives higher results than the rib but is equaled or slightly surpassed in a few cases by the loin. When the fat is entirely eliminated, as shown in the third table, the difference is still less. However, in only one case does the rib cut (steer 18, coagulable nitrogen) give as high results as the round and rump cut; and in another case (steer 503, amido-acid nitrogen) as high as the loin. In six cases the loin cut gives higher results than the round and rump cut. In general, steer No. 505 gives the highest results, especially in the case of the amido-acid nitrogen, there being only one exception, namely, the coagulable nitrogen in the rib cut on the fat-free basis. A further discussion of these results will not be attempted at this time.

For the purpose of making an extended study of the composition of beef extract there was prepared at the Missouri station (at the time of slaughtering) a cold-water extract *a* from a 5-kilo sample of the round of each animal. The filtered extract was coagulated upon the water bath, filtered, and concentrated with one or two filtrations as the concentration proceeded. The extracts have been finally concentrated to a semisolid mass, in which condition they appear to remain in a state of perfect preservation. The life history of the animals from which these extracts have been prepared is known and during the next year cooperation in the examination of these extracts will be requested, to determine the composition of pure beef extract and to learn to what extent variation may be expected.

The president announced the following membership for Committee B on recommendation of referees: Messrs. B. B. Ross, R. W. Thatcher, A. S. Mitchell, Paul Collins, and W. D. Bigelow.

The following committee was appointed to wait upon the Secretary of Agriculture and the Assistant Secretary and invite them to address the convention: Messrs. M. E. Jaffa, J. M. Bartlett, and W. A. Withers.

On motion by Doctor Wiley, the vote on the amendments to the constitution was made special order for 12 o'clock, or following the presidential address, on Friday.

## REPORT ON PRESERVATIVES.

By W. D. BIGELOW, *Referee*.

### SALICYLIC ACID.

#### RAPID DETERMINATION OF SALICYLIC ACID.

The methods of the association for the quantitative determination of salicylic acid are long and tedious because of repeated extraction with immiscible solvents. An attempt was made to simplify these methods by extracting a certain volume of the food, or an aqueous extract thereof, by means of a definite volume of solvent, evaporating to dryness an aliquot portion of the solvent used, and determining the salicylic acid in the residue. The total amount can then be calculated by a factor to be determined by experimental work.

It is, of course, necessary that the solvent, under certain conditions to be adopted, should extract a uniform amount of salicylic acid uncontaminated by substances that

<sup>a</sup> Trowbridge and Grindley, J. Amer. Chem. Soc., 1906, 28: 472.

would interfere with the reaction by which the salicylic acid should finally be estimated in the residue. Owing to its rapidity and convenience, the ferric chlorid reaction has usually been employed for determining the amount of salicylic acid present. It is, therefore, important that the solvent employed should not extract tannin from the food.

In order to determine what solvents should be most advantageously employed as far as delicacy of reaction and freedom from tannin or other interfering bodies is concerned, Mr. Charles S. Ash extracted 50 cc portions of claret containing salicylic acid in amounts varying from 0.025 mg to 0.5 mg and treated the residue obtained by evaporation of the solvent with ferric chlorid in the usual manner. The results are given in the following table:

*Comparative efficiency of solvents on salicylic acid dissolved in 50 cc of claret (Ash).*

Milli-grams salicylic acid present.	Ether.	Chloroform.	Ether and petroleum ether.	Dichloroacetylene.	Trichloroacetylene.	Ether and hydrogen peroxid.	Ether. residue extracted with—		Petroleum ether.	Carbon bisulphid.	Carbon tetrachlorid.	Toluene.
							Carbon tetrachlorid.	Petroleum ether.				
0.500	None.	Good.	Faint trace.	Good..	Good..	Good..	Good.	Good....	Faint trace.	Good.	Good..	Good.
.250	.....	Good.	None..	Fair..	Good..	Faint	Good.	Faint....	None..	Good.	Good..	Good.
.100	.....	Good.	.....	Trace	Trace..	Trace	Fair..	Nothing.	.....	.....	Good..	Good.
.050	.....	Good.	.....	Trace?	None..	None..	None.	.....	.....	.....	Good..	Good.
.025	.....	Good.	.....	None..	.....	.....	.....	.....	.....	.....	Trace?	Good trace.

Mr. Ash found that the first five solvents given in the table extract tannin in the order in which they are mentioned—that is, ether extracts the greatest amount and trichloroacetylene the least. The last three solvents—carbon bisulphid, carbon tetrachlorid, and toluene—do not extract tannin, and the ferric chlorid reaction in the residue obtained by them from wine is clear and characteristic of ferric salicylate. It will be noted that the residue from the ether extraction gave no reaction whatever with ferric chlorid. This was due to the presence of tannin, which entirely obscured the reaction. With chloroform much better results were obtained, but even here the reaction was partially obscured by tannin, which was also true of the residue from dichloroacetylene.

The data given in the column headed "Ether and hydrogen peroxid" were determined by oxidizing with ammonia and hydrogen peroxid the residue obtained by evaporating the ether extract. This destroys the tannin and also partially converts benzoates and saccharin when present into salicylic acid. The salicylic acid was then again extracted with ether, the ether extract evaporated, and the residue tested with ferric chlorid. As previously stated, the greatest freedom from interfering substances attended the use of carbon tetrachlorid and toluene, the latter appearing to extract slightly more salicylic acid than the former, and thus affording a better test in the presence of a small amount of that substance. Chloroform was also very satisfactory, being inferior to carbon tetrachlorid and toluene in respect of dissolving interfering substances, though apparently slightly superior in the amount of salicylic acid extracted.

## Extraction of salicylic acid from different wines.

[Salicylic acid found.]

## BY MEANS OF CARBON TETRACHLORID.

Salicylic acid added.	Angelica.			Sherry.			Port.			Claret.			Dry white wine.		
	Colorimetrically.	By weight.	By titration.	Colorimetrically.	By weight.	By titration.	Colorimetrically.	By weight.	By titration.	Colorimetrically.	By weight.	By titration.	Colorimetrically.	By weight.	By titration.
Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.	Mgs.
100	63.2	63.4	60.8	60.6	61.6	60.0	60.2	60.0	49.4	49.6	50.4	51.0	49.6	49.2	49.2
50	30.6	30.6	30.6	30.6	31.6	32.4	29.8	30.4	30.8	24.4	24.8	24.2	24.6	25.4	24.6
25	14.6	14.6	15.2	14.8	15.2	15.8	14.8	14.6	14.8	13.4	11.8	12.0	13.6	12.4	12.4
10	6.0	.....	6.2	6.2	.....	6.0	6.0	.....	6.0	5.2	.....	5.6	.....	.....	5.6
5	3.0	.....	.....	3.2	.....	.....	3.0	.....	.....	2.6	.....	.....	2.4	.....	.....
2.5	1.6	.....	.....	1.6	.....	.....	1.6	.....	.....	.....	.....	.....	.....	.....	.....
0	.....	4.4	1.4	.....	4.8	1.4	.....	5.0	1.4	.....	3.8	0.8	.....	3.8	1.2

## BY MEANS OF TOLUENE.

100	80.0	80.4	79.4	78.0	83.0	80.4	82.0	79.8	79.0	69.4	68.0	71.2	70.0	69.4	70.2
50	40.0	37.6	39.2	39.2	41.0	41.8	40.2	40.4	39.0	34.0	35.8	35.4	35.4	35.4	35.0
25	19.6	19.4	19.4	20.0	20.0	19.8	19.6	18.6	18.4	18.4	16.8	18.0	18.2	18.8	19.2
10	7.8	.....	7.8	8.0	.....	7.8	8.0	.....	8.0	7.4	.....	7.6	7.5	.....	7.4
5	4.0	.....	.....	3.8	.....	.....	4.0	.....	.....	4.0	.....	.....	3.6	.....	.....
2.5	2.0	.....	.....	2.0	.....	.....	2.0	.....	.....	.....	.....	.....	.....	.....	.....
0	.....	6.0	1.4	.....	7.8	2.4	.....	6.4	1.4	.....	5.6	0.8	.....	4.8	1.4

## SHORT METHOD FOR THE QUANTITATIVE DETERMINATION OF SALICYLIC ACID.

From the results of the qualitative test made by Mr. Ash it appeared that it would be advantageous to confine the work with the quantitative method to carbon tetrachlorid and toluene. Accordingly, Mr. Ash applied the method to various types of wine containing known amounts of salicylic acid varying from 2.5 to 100 mg per 100 cc. One hundred cubic centimeters of the wine were acidified with 5 cc of sulphuric acid (1 to 3) and 50 cc of the solvent were added, gently but thoroughly mixed, and the solvent separated after centrifuging; 25 cc of the solvent were transferred to a weighed watch glass by means of a pipette. With toluene the best results were obtained using a watch glass 4.5 inches in diameter and with carbon tetrachlorid one 4 inches in diameter.

The solvent was allowed to evaporate spontaneously and the amount of residue determined by weighing. The residue was then dissolved in 5 cc of neutral alcohol and transferred into a small casserole, the watch glass being washed thoroughly with neutral boiling water and the salicylic acid titrated with one-hundredth normal barium hydroxid, 1 cc of the reagent being equal to 1.38 mg of salicylic acid.

An aliquot part of the solvent was allowed to evaporate spontaneously, the residue dissolved in 2 or 3 cc of alcohol and diluted with water sufficiently for the colorimetric determination with ferric chlorid. When the amount of salicylic acid present in the original sample was not less than 25 mg per 100 cc, the results obtained by weighing and titration were far superior to those obtained by the colorimetric method, but with smaller amounts the last method was the only one applicable. No tannin was found in any of the residues and the ferric chlorid reactions were clear and entirely characteristic of pure salicylic acid.

In the gravimetric and volumetric determination small amounts of soluble substances were extracted by the solvent. The weight of the residue from 25 cc of the solvent used in extracting normal wine varied from 2 to 3 mg and its acidity was equal to about 0.5 cc of one-hundredth normal barium hydroxid. The results obtained by Mr. Ash on different types of wine are given in the table. In each case these results are the average of three closely agreeing determinations.



It will be noted that the results obtained by Mr. Ash with each solvent and with each type of wine are entirely consistent, and the results obtained by weighing and by titrating the residue agree closely with each other. For instance, by means of carbon tetrachlorid approximately 50 per cent of the salicylic acid present is extracted from dry, red, and white wines. The same reagent, however, extracted slightly more than 60 per cent of the salicylic acid present in the sherry, port, and angelica. Toluene, on the other hand, was found to extract about 70 per cent of the salicylic acid present in dry, white, and red wine and about 80 per cent of that present in sherry, port, and angelica. It would appear that this method might be used advantageously at least for a preliminary determination of the amount of salicylic acid present.

The method was further examined by the referee and by P. B. Dunbar with a view to determining the reason for the varying results obtained with the different types of wine and so modifying the method that uniform results with all substances might be obtained. This investigation was confined entirely to carbon tetrachlorid because of the noninflammability of that substance. Known amounts of salicylic acid were dissolved in dilute alcohol, varying in concentration from 5 to 50 per cent by volume. One hundred cubic centimeters of these dilute solutions of alcohol, containing 1 mg of salicylic acid per cubic centimeter, were shaken in a separatory funnel with 50 cc of carbon tetrachlorid and the amount of salicylic acid determined in 25 cc of the solvent. The figure so obtained was multiplied by two for the purpose of determining the percentage of the salicylic acid extracted in the total amount of solvent employed. The following results were obtained:

Alcohol by volume.	Salicylic acid recovered.	Alcohol by volume.	Salicylic acid recovered.
<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
5.....	44.0	25.....	64.0
10.....	50.0	30.....	64.5
15.....	60.0	40.....	58.6
20.....	61.8	50.....	46.0

It will be noted that the percentage of salicylic acid recovered under these conditions increases with the alcoholic content of the solution up to 25 per cent and then decreases, the maximum results being obtained with from 25 to 30 per cent of alcohol. California red wines, dry and sweet, were then treated in the same manner. Their alcoholic content was increased to 25 per cent and salicylic acid was dissolved in them to the extent of 1 mg per cubic centimeter. Several determinations of salicylic acid were then made by the method described and in each instance 61.2 per cent of the amount of salicylic acid added was recovered. The method was also applied to 100 cc of a solution containing 10 grams of sugar and 25 per cent of alcohol by volume. The amount of salicylic acid recovered from this solution was practically identical with that recovered from a 25 per cent solution of alcohol. Blanks were also run by extracting with carbon tetrachlorid 20, 30, 40, and 50 per cent alcohol acidified with 5 cc of sulphuric acid (1 to 3). It was found that no sulphuric acid was extracted and it was therefore unnecessary to wash the carbon tetrachlorid solution with water after extraction.

#### DETERMINATION OF SALICYLIC ACID IN DARK BEER AND OTHER CAMELIZED SUBSTANCES.

Attention has frequently been called to the possibility of error in the determination of salicylic acid in malt extract and beer prepared from highly colored malt and in highly caramelized substances, such as certain varieties of breakfast food.<sup>a</sup> This

<sup>a</sup> J. Brand, *Zts. gesam. Brauw.*, 1893, 16: 303; H. Kiliani and M. Bazlen, *Berichte*, 1894, 27 (3): 3115-20; *Amer. Brewer's Rev.*, 1907, 21 (5): 222; *Western Brewer* 1907, 32 (8): 456.

matter was independently studied by A. M. Doyle and P. B. Dunbar, of the Bureau of Chemistry. Both reported that the color given by ferric chlorid with the material extracted from highly colored malt by ether was quite different from the salicylic acid and one should not be mistaken for the other, although the presence of a small amount of salicylic acid may readily be masked by the material extracted from highly colored malt and similar material.

Experiments on malt-nutrine alone and on malt-nutrine containing salicylic acid (100 mg per liter) indicate that there is some possibility of being deceived by the color when ferric chlorid is added directly to the dish containing the dried residue obtained by evaporating the ether solution. In this case a color is sometimes developed which slightly resembles the salicylic acid reaction. If this color is examined in a good light and compared with the color developed by salicylic acid and ferric chlorid there is little danger of being deceived. It is better, however, to carry the evaporation of the extract to about 5 cc on the steam bath and then to complete the evaporation by means of a blast of air, since heating to dryness may darken the residue. The dry residue should be dissolved in a little hot water and ferric chlorid added to this solution. Under these conditions it seems impossible to mistake the salicylic acid reaction. Millon's reagent, freshly prepared, which has been suggested for the detection of salicylic acid in such substances, was not found by either Miss Doyle or Mr. Dunbar to be as satisfactory as ferric chlorid. In the absence of salicylic acid this reagent gives a light pink color, whereas in its presence a deep red is given. The intensity of the color seems to vary so much with the time of boiling, however, as to render the reaction uncertain and unsatisfactory. Both methods were also applied to highly caramelized breakfast foods, to which these observations also apply.

#### DETERMINATION OF BENZOIC ACID.

Several methods for the quantitative determination of benzoic acid have been suggested recently. Among these the following have been studied by the referee and his collaborators: (1) La Wall's method; (2) La Wall's method modified by mixing a definite weight of tomato ketchup with sufficient saturated sodium chlorid solution to make a definite volume, filtering, and extracting an aliquot portion of the filtrate with chloroform; (3) precipitation as copper benzoate; (4) precipitation as silver benzoate; (5) distillation with steam after decomposing organic matter with sulphuric acid and extracting with ether. It will be noted that the first four methods depend on extracting the benzoic acid from the food or an aliquot extract of the food by means of an immiscible solvent, whereas the fifth depends on separating the preservative from the food by distilling with steam.

Before applying the first four methods a preliminary study was made of the relative advantages of several solvents. This question has been greatly altered by the introduction of the principle of "salting out" the benzoic acid by means of a saturated solution of sodium chlorid. The benzoic acid is thus rendered much less soluble in the solution from which it is extracted and consequently much more readily extracted by the immiscible solvent. The relation of the immiscible solvent to the preservative thus more nearly coincides with the direct solubility of the former in the latter. An approximate determination was therefore made of benzoic acid in several solvents. It was found that the solvents more commonly employed dissolved benzoic acid as follows, 100 cc of the solvent being used in each case: Ether, 25.70 grams; chloroform, 17.34 grams; carbon tetrachlorid, 7.65 grams; toluene, 7.58 grams; benzol, 6.40 grams.

The chief desiderata in the extraction of a substance of this kind are, first, completeness of extraction; second, freedom of the extract from interfering substances; and third, noninflammability. Of the four solvents mentioned, it would appear from the solubility that by far the most complete extraction can be obtained by means of ether, and this is known to be true. Ether is objectionable, however, because of its property of dissolving water and its consequent tendency to extract tannin, salts, min-

eral acids, and other interfering substances. The use of ether is also a source of danger because of its great inflammability.

Chloroform has always been found inapplicable to the extraction of benzoic acid because of the incompleteness of the extraction so effected. Of the solvents studied, however, it stands second to ether in its power of dissolving benzoic acid. A study was therefore made of the efficiency of chloroform in extracting benzoic acid in a saturated sodium chlorid solution, and the extraction was found to be practically complete.

In order to determine the efficiency of chloroform with respect to the extraction of interfering substances, 500 cc of saturated sodium chlorid solution were acidified with 5 cc of sulphuric acid (1 to 5), and extracted with four portions of chloroform containing 100, 50, 50, and 25 cc, respectively. Each portion of chloroform was removed as completely as possible from the solution, the four portions mixed and distilled on a hot plate to a low volume, the last being removed at ordinary temperature in a current of dry air. The residue left by evaporating the chloroform was not weighable. It was dissolved in water and titrated with tenth-normal alkali. Duplicate determinations required 0.03 and 0.04 cc of tenth-normal alkali for neutralization, thus indicating that the error, owing to the extraction of mineral acids under the conditions noted, is not greater than 0.6 mg of sodium benzoate.

One-half gram of tannin was then dissolved in 500 cc of saturated salt solution and extracted with chloroform as just described. The residue left by evaporating the chloroform was not weighable, but required in the duplicate determinations 0.02 and 0.04 cc of tenth-normal alkali for neutralization, which is equivalent to 0.5 mg of sodium benzoate. One-half gram of tannin and 5 cc of sulphuric acid (1 to 5) were then added to 500 cc of saturated sodium chlorid solution and extracted with chloroform as described. The residue obtained by extracting with chloroform was not weighable, duplicate determinations requiring 0.05 and 0.07 cc of alkali, respectively, for their neutralization, equivalent to 0.9 mg of sodium benzoate.

The presence of acetic acid in the ketchups did not appear to cause any inaccuracy. In order to determine whether a large amount of acetic acid would lead to erroneous results, 1 cc of 99.5 per cent acetic acid was dissolved in 200 cc of chloroform and various portions dried, as in the case of the chloroform extract from the samples of ketchup, and the residues titrated. Ten cubic centimeters of the chloroform solution without evaporation require 9 cc of tenth-normal alkali to neutralize it, equivalent to 0.052 gram of acetic acid, or to 0.141 gram of sodium benzoate. Ten cubic centimeter portions of the solution were evaporated before an air blast until no liquid was visible, and 10 cc of water were added to the dish and titrated. An alkaline reaction was secured on the addition of 0.2 cc of one-hundredth-normal alkali. When 50 cc portions were evaporated in the same way and 10 cc of neutral water added to the dish 0.2 cc of one-hundredth-normal alkali were required to give an alkaline reaction to phenolphthalein. When 10 cc portions were evaporated until the chloroform had apparently disappeared but a few drops of liquid remained. The odor of acetic acid was very apparent and the residue required 0.2 cc of tenth-normal alkali for neutralization. Ten cubic centimeter portions were evaporated until the chloroform had apparently disappeared, but still contained a slight amount of liquid with a strong odor of acetic acid. The residue was placed overnight in a sulphuric acid desiccator, 10 cc of water were added to the dish and titrated as above, requiring 0.1 cc of one-hundredth-normal alkali to give an alkaline reaction. It is apparent that while acetic acid will be extracted by the chloroform, no error is occasioned if care be taken to dry the residue either in a current of air or in a desiccator. It was also found that no appreciable error was caused by the presence of tartaric or citric acid. Lactic acid, when present, was extracted to a considerable extent by chloroform, but after being evaporated to dryness before an air-blast the error amounted to only 0.02 per cent.

If the volume of salt solution taken in this work is equivalent to 100 grams of food extracted as in the case of the subsequent studies, the error caused by the extraction of interfering bodies by means of chloroform is less than 0.001 per cent, and is negligible. It would appear, therefore, that chloroform possesses the following advantages: First, after saturating a solution with sodium chlorid a practically complete extraction of benzoic acid may be made; second, the benzoic acid extracted is not accompanied by other bodies that interfere with its determination by means of titration; third, the solvent is not combustible; fourth, it is inexpensive. In connection with subsequent work on the determination of benzoic acid, therefore, no study was made of any other solvent than chloroform except as described under Method IV. The detail of the methods studied is as follows:

#### LA WALL AND BRADSHAW METHOD (METHOD I).

This method was described in full by the authors in the *American Journal of Pharmacy* (volume 80, pages 171-172). The principle depends upon the method outlined by F. X. Moerk in an article published in the *Proceedings of the Pennsylvania Pharmaceutical Association* for 1905, page 181. The details of the method are as follows:

To 20 grams of the substance under examination add 2 grams of sodium chlorid, 5 cc of hydrochloric acid, and 25 cc of a saturated solution of sodium chlorid. Shake thoroughly for five minutes, transfer to a moistened filter, and wash with a saturated solution of sodium chlorid until 100 cc of the filtrate are collected. Transfer the filtrate to a separatory funnel and shake with three portions of chloroform, using 25, 15, and 10 cc respectively. Evaporate the chloroform at room temperature. If the residue is white and crystalline, dry over sulphuric acid in a desiccator and weigh. If yellowish and oily, dissolve in 10 or 15 cc of weak ammonia acidified with dilute sulphuric acid and again extract with chloroform. The residue is dissolved in 3 to 5 cc of neutral alkali and titrated with twentieth-normal alkali solution, using phenolphthalein as indicator. The titration should agree closely with the gravimetric determination, the difference being rarely more than 1 or 2 mg.

As will be seen by a comparison of the figures obtained by various methods given in the table on page 71, the results by weighing the benzoic acid, given under Method I, are much higher than those obtained by titration. All collaborators report difficulty in securing an adequate filtration. F. W. Heyl obtains very satisfactory results by titration, whereas the figures of all other analysts were low. In the Division of Foods the method was applied to the examination of a considerable number of commercial ketchups. With the low-grade product, made from skin and core pulp, filtration was possible though somewhat slow. With many of the high-grade ketchups the material was so finely divided that filtration was almost impossible. In this connection should be considered the limited solubility of benzoic acid in a saturated solution of sodium chlorid to be mentioned subsequently.

#### MODIFICATION OF LA WALL AND BRADSHAW METHOD (METHOD II).

This method is identical with the one just described, except that no attempt is made to obtain a complete filtration.

To 200 grams of ketchup are added 20 grams of finely powdered sodium chlorid in a liter flask, and enough of a saturated solution of sodium chlorid is added to make a liter. The contents of the flask are thoroughly mixed and allowed to stand overnight, when they are filtered, and 500 cc of the filtrate are transferred to a separatory funnel, treated with 5 cc of sulphuric acid (1 to 5) and extracted repeatedly with chloroform, using 100, 50, 50, and 25 cc of chloroform, respectively.

In this extraction a troublesome emulsion was formed which it was found could be broken up to a considerable extent by centrifuging and by stirring with a glass rod. Where a centrifuge is not available much may be accomplished by swinging the separatory funnel with the hand. It is important that after each extraction the chloroform be removed as completely as possible, and at the same time the utmost care must be exercised to prevent any emulsion passing through with the chloroform.

Owing to the mineral acid present in the aqueous liquid, the presence of a slight amount of this emulsion in the chloroform layer causes serious error in a titration of the residue.

The results obtained by this method, both by weighing and by titrating the chloroform residue, are given in the table below, under Method II. It will be observed that in all cases the results obtained by weighing are obviously too high, while the results obtained by titration with this method are on the whole very satisfactory. It would appear therefore that the method is fairly satisfactory for the determination of benzoic acid in tomato ketchup, which presumably offers as great difficulties for this determination as any food to which this preservative is commonly added.

*Determination of sodium benzoate in ketchup by three methods.*

Analyst.	Sodium benzoate added.	Sodium benzoate found.				
		Method I.		Method II.		Precipitation by silver nitrate. <sup>a</sup>
		By weight.	Titrated.	By weight.	Titrated.	
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
W. L. Dubois.....	0.247	0.171	0.166	0.232	0.179	0.166
Do.....	.146	.130	.261	.223	.172	.143
F. W. Heyl.....	.168	.185	.170	.169	.157	.....
Do.....	.168	.185	.170	.163	.155	.....
Do.....	.168	.190	.170	.....	.....	.....
Do.....	.168	.183	.162	.....	.....	.....
Do.....	.168	.169	.154	.....	.....	.....
Do.....	.168	.178	.162	.....	.....	.....
Do.....	.030	.....	.....	.....	.....	.029
Do.....	.060	.....	.....	.....	.....	.056
Do.....	.060	.....	.....	.....	.....	.056
Do.....	.060	.....	.....	.....	.....	.056
Do.....	.060	.....	.....	.....	.....	.059
P. B. Dunbar.....	.247	.....	.....	.270	.244	.238
Do.....	.247	.....	.....	.258	.245	.241
Do.....	.247	.....	.....	.262	.245	.242
Do.....	.247	.....	.....	.277	.250	.250
Do.....	.168	.....	.....	.171	.170	.204
Do.....	.168	.....	.....	.183	.170	.168
Do.....	.168	.....	.....	.196	.169	.172
Do.....	.168	.....	.....	.195	.171	.180
M. C. Albrech.....	.033	.035	.024	.....	.....	.....
Do.....	.033	.036	.030	.....	.....	.....
Do.....	.164	.....	.....	.183	.133	.....
Do.....	.164	.....	.....	.199	.149	.....
Do.....	.164	.....	.....	b. 177	b. 150	.....
Do.....	.164	.....	.....	b. 162	b. 150	.....
C. Conover.....	.060	.....	.....	c. 062	.....	.....
Do.....	.060	.....	.....	c. 062	.055	.....
Do.....	.060	.....	.....	c. 063	.055	.....
Do.....	.060	.....	.....	c. 063	.056	.....
H. L. Schulz.....	.168	.137	.142	.....	.....	.142
Do.....	.168	.147	.140	.....	.....	.....
F. F. Flanders.....	.247	.257	.230	.....	.....	.236
Do.....	.247	.253	.....	.....	.....	.222
Do.....	.168	.176	.148	.....	.....	.139
Do.....	.168	.206	.....	.....	.....	.....

<sup>a</sup> Method IV, pages 74, 75.

<sup>b</sup> 100 grams ketchup diluted with water to 200 cc, mixed, and filtered, 50 cc portions of filtrate, diluted to 100 cc, saturated with sodium chlorid, acidified, and extracted with four portions of 100 cc each of ether.

<sup>c</sup> Same as footnote "a" except extraction is made with chloroform instead of ether, three portions being used of 100, 50, and 50 cc, respectively.

In some cases where the mixture of tomato ketchup and a saturated salt solution was filtered soon after it was prepared, the amount of benzoic acid determined by this method was too low. This suggests the possibility that the solution of the benzoic acid in the saturated salt solution may not have been complete. More satisfactory results were obtained in all cases where the mixture was allowed to stand overnight before filtering.

An approximate determination of the solubility of benzoic acid in a saturated solution of sodium chlorid was made by P. B. Dunbar. In duplicate tests 0.260 and 0.259 gram of benzoic acid were dissolved in 500 cc of saturated solution of sodium chlorid. In another duplicate determination 0.274 and 0.277 gram of benzoic acid were found to be soluble in 500 grams of saturated solution of sodium chlorid to which 5 grams of sulphuric acid (1 to 5) had been added. No special precautions were taken with respect to the temperature of solutions or the purity of the sodium chlorid employed.

It is apparent therefore that a larger amount of benzoate of soda than 0.27 gram can probably not be determined by this method. The fact that a slightly higher amount is reported in some determinations may probably be explained by slightly higher temperature, or slightly lower concentration of the sodium chlorid solution employed.

It is obviously important that the method be so modified as to permit the determination of a larger amount of benzoate of soda. Attempts were made to extract the benzoic acid from the ketchup by means of water, filtering, and saturating an aliquot part of the filtrate with sodium chlorid. The results obtained were not altogether satisfactory, although the amount of work done was not sufficient to permit of definite conclusions. It is probable that by extracting in this manner or with a saturated sodium chlorid solution, previously made slightly alkaline with sodium hydroxid, the method can be so modified as to permit of its application to samples carrying a much higher percentage of benzoate of soda, and at the same time it will be possible to extract a smaller volume of the salt solution.

#### PRECIPITATION WITH COPPER BENZOATE (METHOD III).

Mr. C. S. Ash, of the California Wine Association, and Mr. F. W. Liepsner, of the Division of Foods of the Bureau of Chemistry, studied the determination of benzoic acid by means of precipitation as copper benzoate from the residue left by the evaporation of the solvent. It was hoped that this method might afford a means for determining benzoic acid either alone or in the presence of salicylic acid, but preliminary work showed that while copper acetate is a satisfactory precipitant for benzoic acid when present alone, no precipitate is formed in the presence of salicylic acid in considerable quantity, owing probably to the formation of a soluble double salt.

A series of precipitations of benzoic acid were made in alcoholic solutions of various strengths from 0 to 100 per cent and the curve established, giving the per cent of benzoic acid present that may be precipitated as copper benzoate in various strengths of alcohol. The results are as follows:

#### *Precipitation of benzoic acid with varying strengths of alcohol.*

Per cent of alcohol.	Per cent of benzoic acid precipitated.	Per cent of alcohol.	Per cent of benzoic acid precipitated.
0	83	50	70
10	84	60	65
20	88	70	58
30	86	80	48
40	75	100	0

It appears that the amount of benzoic acid precipitated is somewhat increased by the presence of alcohol up to 20 per cent of the latter. The increase in strength of the alcohol beyond this point decreases the amount of benzoic acid precipitated, at first slowly and then rapidly, the copper benzoate being completely soluble in strong alcohol. All precipitations were therefore made in a solution containing 20 per cent of alcohol by volume.

To further guard against error due to the solubility of the precipitate, the alcoholic solution from which the precipitation was made was saturated with freshly precipitated basic copper benzoate. A stock solution of the alcohol so saturated was made up and used throughout the work. It was found that the results obtained by precipitation in neutral solution were too high and that a slight acidity in the solution was necessary. This was accomplished by adding a little acetic acid to the copper reagent. The solutions employed and the method of determination were as follows:

**Copper acetate reagent.**—A mixture of 250 cc of 95 per cent alcohol and 750 cc of water is saturated with copper benzoate and 2 cc of glacial acetic acid added, followed by a concentrated solution of sodium benzoate in 20 per cent alcohol to form a small amount of permanent precipitate. This solution is filtered just before using.

**Alcohol solution.**—A mixture of 1 part of 95 per cent alcohol and 3 parts of water is saturated with basic copper benzoate, prepared by precipitating copper acetate solution with sodium benzoate, filtering, and washing. An excess of the basic copper benzoate is added to the alcohol in order that additional alcohol may be added from time to time.

**Determination.**—The residue obtained by the evaporation of the chloroform extract is dissolved in a small amount of the alcohol saturated with copper acetate mentioned above and washed into a small beaker with the same solution, 25 cc of the copper acetate solution is added, the mixture stirred, allowed to stand for one hour, filtered on a gooch, and the precipitate washed with the alcohol solution saturated with basic copper benzoate. The solution is then dried and weighed as usual. If preferred, the residue may be washed into a 100 cc flask with the alcohol solution described, the copper acetate reagent added, and the mixture brought up to mark with the alcohol saturated copper benzoate. The whole is then mixed and, after standing one hour, filtered, and the copper determined volumetrically by any of the standard methods.

The following table shows the results obtained, working on a known amount of benzoic acid in aqueous solution. As before stated above, this precipitate consists of basic copper benzoate, represented by the formula  $C_6H_5COO-Cu-OH$ .

*Benzoic acid recovered from an aqueous solution by Method III.*

Benzoic acid present.	Benzoic acid recovered by—	
	Gravimetric method (as copper benzoate).	Titrating excess of copper.
Grams.	Per cent.	Per cent.
0.04	83.5	84.1
.04	84.5	75.4
.05	97.9	97.9
.05	100.0	97.9
.08	101.5	-----
.08	100.5	-----
.10	101.0	99.1
.10	102.8	104.2
.14	102.0	100.5
.14	102.5	100.5
.20	101.5	103.6
.20	101.8	105.1

An attempt was made to apply this method to the determination of benzoic acid in ketchup. A number of samples were treated as follows:

Three hundred grams were made up to 1,500 cc with saturated salt solution, allowed to stand overnight, and filtered; 500 cc of the filtrate were acidified with sulphuric acid and extracted with 3 portions of chloroform of 100, 50, and 50 cc. The extract was evaporated in a current of air, dried over sulphuric acid, the residue weighed, taken up in neutral alcohol, and titrated with tenth-normal sodium hydrate. The solution was then evaporated to dryness and benzoic acid determined gravimetrically, as described above, by use of the copper precipitation method.

The results obtained by this method are as follows:

*Determination of known amounts of benzoic acid in ketchups by Method III.*

Sodium benzoate present.	Sodium benzoate found by titration.	Sodium benzoate found by copper precipitation.
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>
0.258	0.229	0.116
.359	.322	.167
.127	.101	No precipitate.
.431	.392	.183
.198	.149	No precipitate.
.387	.328	.174
.107	.079	No precipitate.
.352	.314	.222
.332	.288	.167
.251	.230	.138
.210	.193	.126
.103	.082	No precipitate.
.355	.303	.150
.307	.286	.152
.324	.277	.142
.146	.119	No precipitate.

An examination of this table shows that when there was present less than 0.15 gram of sodium benzoate no precipitate was formed, and in such cases as did give a precipitate all results were from 0.1 to 0.15 gram low. This seemed to indicate that there was something in the ketchup extract which held back the precipitation and showed conclusively that the method could not be used for such materials in combination with present extraction methods.

It appeared probable that since the completeness of precipitation varied with the alcoholic strength it might be interfered with by sugars, higher alcohols, oils from spices, etc., and the following experiments were performed. The alcoholic extract from 1 gram of spice was added to solutions containing 0.1 gram of sodium benzoate and to blanks. Also 0.1 gram of sugar, glycerin, and dextrin were added to similar solutions with the following results:

*Determination of sodium benzoate in the presence of spices, sugars, etc., by Method III.*

Substance added.	Sodium benzoate present.	Sodium benzoate found.	Substance added.	Sodium benzoate present.	Sodium benzoate found.
	<i>Gram.</i>	<i>Gram.</i>		<i>Gram.</i>	<i>Gram.</i>
Allspice.....	0.1	0.0741	Sugar.....	0.1	0.0941
Cinnamon.....	.0	.0576	Glycerin.....	.0	None.
Cloves.....	.1	.055	Dextrin.....	.1	.0955
Black pepper.....	.0	.0511	Check.....	.0	None.
Red pepper.....	.1	.1621		.1	.0854
	.0	.0809		.0	.....
	.1	.0634		.1	.0977
	.0	.0477			
	.1	.1293			
	.0	.1478			

These figures show that it is the presence of the spices which causes the failure of this method for the determination of benzoic acid in such products. It is possible that it may be applicable to other materials.

PRECIPITATION AS SILVER BENZOATE (METHOD IV).

This method was suggested and elaborated by Mr. W. E. Hillyer.

The sample is extracted by means of ether as directed in Bulletin 107, page 179, or by the method given by Dubois for the extraction of ketchups.<sup>a</sup> The amount

<sup>a</sup> J. Amer. Chem. Soc., 1906, 28: 1616.



of substance used should be such that the portion subsequently extracted with ether will contain approximately 0.1 gram of sodium benzoate. The ether extract, after washing with water, is allowed to evaporate to dryness spontaneously, or the first portion of the ether may be distilled and recovered. After drying completely the residue is taken up with a small amount of absolute alcohol, for the purpose of separating interfering substances as far as possible, and filtered into a small beaker. The alcohol is neutralized with sodium hydroxid, evaporated to dryness, and redissolved in a few cubic centimeters of alcohol saturated with silver benzoate. The solution is filtered if not clear, washed with a few drops of aldehyde-free alcohol, saturated with silver benzoate, and treated with from 10 to 15 cc of a saturated solution of silver nitrate in aldehyde-free alcohol. The precipitate is collected in a gooch, care being taken that the asbestos filter be so constructed as to afford as rapid filtration as possible. The precipitate is then heated in a water-jacketed oven until the ether is driven off, cooled, and weighed.

Care must be taken to perform all operations as quickly as possible in order to prevent the separation of silver oxid. The aldehyde-free alcohol mentioned above is about 95 per cent by volume, and is prepared according to the directions given in Bulletin 107, page 96, with the additional precaution of distilling over soda after treatment with meta-phenylene-diamin hydrochlorid. This method involves the use of a considerable quantity of ether, which is objectionable because of its inflammability and the tendency to dissolve sodium chlorid and other interfering substances. Notwithstanding this, very satisfactory results are reported by Messrs. Hillyer and Flanders in the following table. No other results obtained by this method, using ether as solvent, were reported, though the precipitation of benzoic acid as silver acetate, using chloroform as a solvent, was included in the work of several other collaborators.

*Determination of benzoic acid as silver benzoate in tomato ketchup (Hillyer and Flanders).*

Sodium benzoate added.	Sodium benzoate found.	Sodium benzoate added.	Sodium benzoate found.
<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
0	0	0.030	0.036
0	.004	.030	.056
0	.001	.030	.059
0	0	.146	.101
0	.003	.146	.127
0	.003	.100	.100
0	.001	a. 247	a. 232
.030	.029	a. 247	a. 222
.060	.056	a. 108	a. 159

<sup>a</sup> Last three results by F. F. Flanders; others by W. E. Hillyer.

These results were obtained by extracting and precipitating as silver benzoate in the ether residue following Method II as given on page 70. The figures seem to be in every way comparable with those obtained by Method II (see p. 71). Extraction with ether appears to be much less satisfactory than extraction with chloroform, owing to the removal of interfering substances by the solvent. These bodies are partially removed by means of absolute alcohol, but this introduces an additional operation and the results obtained are not as satisfactory as by extracting with chloroform from a solution saturated with sodium chlorid. In the table comparing Methods I and II (p. 71), is given the percentage of sodium benzoate precipitated as silver benzoate from the residue from Method II; that is, the liquid titrated under Method II was evaporated to dryness and used as a starting point for the silver benzoate method.

In the following table are given the results obtained by the examination of a number of samples of commercial ketchups using this method. In all cases the benzoic acid was extracted by chloroform from a saturated sodium chlorid solution. Here, again, it will be seen that the results obtained by weighing the residue are in all cases slightly

higher than those given by titration, whereas the amounts determined by precipitation as silver benzoate are almost identical with the amount obtained by titrating the chloroform residue. This method is evidently worthy of further study. It is much more tedious than Method II, but is of value for the purpose of checking the results obtained by that method when a further confirmation seems desirable.

*Determination of benzoic acid in commercial samples of tomato ketchup by precipitation as silver salt from chloroform extract.*

Analyst.	Chloroform residue weighed.	Chloroform residue titrated.	Silver precipitate.	Analyst.	Chloroform residue weighed.	Chloroform residue titrated.	Silver precipitate.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
C. P. Wilson.....	0.30	0.28	0.28	P. B. Dunbar.....	0.20	0.17	0.18
Do.....	.11	.10	.09	Do.....	.28	.26	.26
Do.....	.19	.16	.15	Do.....	.09	.08	.07
Do.....	.16	.14	.14	Do.....	.21	.19	.18
Do.....	.20	.17	.16	Do.....	.20	.17	.18
Do.....	.33	.32	.31	Do.....	.17	.15	.15

These samples were treated according to Method II, given on page 70. The residue obtained by the evaporation of the chloroform extract was first weighed, then dissolved in about 5 cc of neutral alcohol, the solution so obtained diluted with water and titrated with saturated alkali solution. This solution when exactly neutralized is evaporated to dryness, after which the benzoic acid was determined by precipitation as silver benzoate.

#### THE DISTILLATION OF BENZOIC ACID FROM SULPHURIC ACID SOLUTION (METHOD V).

This method was suggested and elaborated by Mr. R. M. West,<sup>a</sup> and depends on the distillation of benzoic acid with steam after the addition of sufficient concentrated sulphuric acid to insure the complete charring of vegetable tissue and prevent volatilization of coloring matter and oil. The distillation is conducted by means of a flask shown on page 21, the procedure being as follows:

About 10 grams of the sample are weighed into the inner flask of the apparatus, 1.5 to 2 grams of paraffin added, and the flask connected with the condenser. Ten cubic centimeters of strong sulphuric acid are added through a drop funnel at a rate sufficient to complete the addition at from two to three minutes, the flask is gently agitated, to mix the contents thoroughly, and allowed to stand from five to ten minutes after all apparent action of the sulphuric acid has ceased. About 150 cc of distilled water are placed in the outer flask of the apparatus and the water slowly brought to a boil and the boiling continued until 100 cc of the distillate have been collected. The stopcock in the outer flask is left open until the water has heated sufficiently to prevent the contents of the inner flask being drawn into the outer flask.

The distillate is filtered into a separatory funnel and the original receiver and filter are washed with two portions of water of about 10 cc each. The distillate is then extracted with three portions of ether of 50, 30, and 20 cc, respectively. The combined ether extracts are washed repeatedly with water until a 25 cc portion requires not more than 0.10 cc of decinormal alkali for neutralization. The ether extract is then distilled to small volume, after which it is evaporated before a blast of air, dried in a desiccator to constant weight and weighed. The residue is also dissolved in neutral alkali, using phenolphthalein as indicator.

The results obtained by titration agree closely with those obtained by weighing. Excessive foaming is likely to occur when the steam begins to pass into the inner flask. This may be caused by distilling too soon after the addition of the acid, by an insufficient amount of paraffin, or by an unusual amount of sugar in the ketchup. Care must be exercised to prevent the foam passing into the condenser.

<sup>a</sup> J. Ind. and Eng. Chem., 1909, 1: 190.

The distillation should be conducted at such a rate that 100 cc of the distillate may be obtained in from twenty-five to thirty-five minutes. Occasionally some paraffin is carried over mechanically, and this may usually be removed from the surface of the distillate by means of a wire or glass rod.

The following results were obtained on ketchups containing a known amount of sodium benzoate:

*Determination of sodium benzoate in ketchup by Method V (West).*

Sodium benzoate added.	Sodium benzoate found.	
	By weight.	By titration.
<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
0.00	0.01	0.01
.10	.09	.10
.25	.25	.24
.50	.47	.47

THE DETECTION OF CINNAMIC ACID.

The statement has frequently been made that cinnamic acid is being used for the preservation of foods, especially in the case of tomato ketchup. The claim has often been made by those interested in the preservation of ketchup with benzoic acid that the presence of cinnamic acid could not be detected and that firms claiming to use no preservative were preserving with that substance. Two qualitative methods for the detection of cinnamic acid, differing slightly from each other, were elaborated by P. B. Dunbar. Both of these methods depend upon the well-known fact that cinnamic acid is oxidized to benzaldehyde by dilute chromic acid mixture.

*Method 1.*—One hundred grams of ketchup were treated with 100 cc of water and 5 cc of sulphuric acid (1 to 5) and the mixture extracted directly with three portions of chloroform, using 50, 25, and 25 cc, respectively. The chloroform extract was made alkaline with ammonia and evaporated to dryness on the water bath. The residue was dissolved in a small amount of hot water, filtered, again evaporated to dryness, and heated to boiling with 5 cc of dilute chromic acid mixture (1 part of dilute sulphuric acid saturated with potassium bichromate and 7 parts water). The odor of benzaldehyde is strongest when the mixture is cooled until the fumes of sulphuric acid are no longer apparent.

*Method 2.*—Two hundred grams of the ketchup are diluted to 500 cc with water, allowed to settle, and filtered. An aliquot portion of the filtrate, 250 cc or more, is acidified with 5 cc of sulphuric acid (1 to 5), extracted with chloroform, and the remainder of the operation conducted as under Method 1.

The second method appears to be slightly more delicate than the first, although with either it was possible to detect cinnamic acid in tomato ketchup when present to an extent of 25 mg per kilogram.

This reaction is also given by cinnamic aldehyde. The method, therefore, does not distinguish of itself between cinnamic aldehyde, resulting from the use of cinnamon as a flavor and cinnamic acid used as a preservative, except that the amount of cinnamic aldehyde present in the commercial ketchups examined was not sufficient to give a reaction. If cinnamic acid were present in the ketchup, it would be detected by the methods used for the detection of benzoic acid. Cinnamic aldehyde, on the other hand, would not be detected by the methods suggested for benzoic acid. The benzoic-acid residue obtained by the evaporation of the chloroform extract may be examined by the cinnamic-acid methods described.

The germicidal and antiseptic properties of cinnamic acid were investigated by G. W. Stiles, who found them to be very much lower than those of benzoic acid. The preservation of a food, therefore, would require a much larger percentage of cinnamic

acid than benzoic acid. In fact, the antiseptic properties of a saturated solution of cinnamic acid are so slight that this substance would probably not serve as a preservative for foods.

A method for the separation of benzoic acid and cinnamic acid by precipitation of the latter with manganous salts<sup>a</sup> was tried unsuccessfully by Mr. Dunbar, who was unable to secure a precipitation of either benzoate or cinnamate of manganese in dilute solution. As is to be expected, Mohler's and Peter's reaction also give the same end reaction in the presence of cinnamic acid.<sup>b</sup>

## REPORT ON TEA, COFFEE, AND COCOA.

By A. G. WOODMAN, *Associate Referee.*

The work of the referee for the past year has been limited to a study of methods for the determination of caffeine and caffetannic acid in coffee, extract in tea, crude fiber and starch in chocolate, and sugars in milk chocolate. Twenty-two samples were prepared and sent out to those who had expressed a willingness to collaborate, ten on tea and coffee and twelve on cocoa products. These were accompanied by the following directions and a letter of transmittal:

### CAFFETANNIC ACID.

(a) *Krug's method.*—Proceed as directed in Bul. 107, p. 155. (Note that the formula for lead caffetannate should be  $Pb_3(C_{15}H_{15}O_8)_2$  as in Bul. 107, Rev.) Save the filtrate for the determination of caffeine. After weighing the lead caffetannate determine its lead content as follows: Digest with aqua regia, add sulphuric acid, heat to fumes, cool, dilute, add alcohol, settle, filter, ignite, and weigh as lead sulphate. Calculate as per cent of lead.

(b) *Method of Trillich and Göckel.*<sup>b</sup>—Boil 3 grams of coffee one-half hour with water, filter, and repeat this treatment on the residue three times. The united filtrates are made up to 1,000 cc. To 400 cc add 1 cc of basic lead acetate solution and allow to stand overnight. Filter, wash, decompose the precipitate with sulphuretted hydrogen, filter from lead sulphid, evaporate to dryness, and weigh.

### CAFFEIN.

In the filtrate from the lead caffetannate precipitate the lead with hydrogen sulphid, filter, and remove the excess of hydrogen sulphid by boiling, concentrating the solution, if necessary, to about 100 to 150 cc. Add tenth-normal potassium iodid solution of iodine in excess, filter through a little glass wool and determine the excess of iodine with tenth-normal sodium thiosulphate.

1 cc tenth-normal iodine equals 0.00485 gram caffeine.<sup>c</sup>

### EXTRACT IN TEA.

(a) Follow the provisional method as described in Bul. 107, p. 149.

(b) Follow the method proposed by Doolittle and Woodruff (Bul. 105, p. 48).

### CRUDE FIBER (SAMPLE A).

Proceed as directed in Bul. 107 under "VI. General Methods," 11, page 56, except that the fiber is filtered and weighed on a paper. The sample should be pulverized by grinding with ether as described in the succeeding paragraph.

### CRUDE STARCH (COPPER-REDUCING MATTERS BY DIRECT ACID HYDROLYSIS), SAMPLE A.

Weigh 4 grams of the material if unsweetened, or 10 grams if sweetened, into a small wedgewood mortar, add 25 cc of ether and grind with a pestle. After the coarser material has settled decant off the ether together with the fine suspended matter on

<sup>a</sup> Scoville, *Amer. J. Pharm.*, 1907, 79 [12]: 549-551.

<sup>b</sup> *Zts. Nahr. Genussm.*, 1898, 101.

<sup>c</sup> Gomberg: *J. Amer. Chem. Soc.*, 1896, 18: 331.

a 11 cm, blue ribbon, S. and S. paper. Repeat this treatment until no more coarse material remains. After the ether has evaporated from the filter, transfer the fat-free residue to the mortar by means of a jet of cold water and rub to an even paste, filtering on the paper previously employed. Repeat this process until all sugar is removed. In the case of sweetened products the filtrate should measure at least 500 cc. Conduct the hydrolysis of the residue as directed for "Starch" under "VI. General Methods," 8 (a), page 53, Bul. 107, Rev., except that after neutralizing with sodium hydroxid, add 5 cc of basic lead acetate solution (prepared as directed under "VI. General Methods," 6 (b), (1), page 40) before completing the volume to 250 cc. To 100 cc of the filtrate add 1 cc of 60 per cent sulphuric acid, filter off the lead sulphate and determine reducing matters in 25 cc of the filtrate as directed under "VI. General Methods," for Reducing Sugars, 7, (b), (2), page 49. Determine copper by the direct weighing of cuprous oxid, 7, (c), (6), page 53.

## SUGARS (SAMPLE B).

Determine the lactose and sucrose as described by Dubois.<sup>a</sup>

The amount of work requested was purposely made small in order that it should not prove burdensome, but in spite of this results were received from only four chemists, two on cocoa products and two on tea and coffee.

## TEA AND COFFEE.

The results on tea and coffee are shown in the following table:

*Cooperative work on coffee and tea.*

Analyst.	Caffetannic acid.		Caffein.	Extract in tea.	
	Krug method.	Trillich and Göckel method.		Krauch method.	Doolittle and Woodruff method.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
F. O. Woodruff, New York.....	12.65	14.42	0.38	.....	52.95
	12.41	17.10	.33	.....	51.50
	11.05	15.26	.29	45.64	51.69
	12.17	16.26	.53	(Whole)	50.92
	11.25	14.28	.38	40.74	51.42
	11.49	16.09	.....	(Ground)	45.90
	12.27	.....	.....	.....	.....
	12.23	.....	.....	.....	.....
	12.27	.....	.....	.....	.....
	12.28	.....	.....	.....	.....
A. G. Woodman, Boston.....	11.19	8.03	.68	39.60	49.72
	13.21	7.80	.75	42.15	50.16
	.....	8.26	.88	40.82	50.28
	.....	8.22	.74	.....	50.99
	.....	.....	.78	.....	.....
Mark Millikin, Hamilton, Ohio.....	11.09	.....	.81	.....	.....
	11.86	9.33	.67	.....	44.8
	10.11	8.8	1.06	.....	42.7

## COMMENT BY MR. WOODRUFF.

*Krug method for caffetannic acid.*—(1) The water must be kept to constant volume during thirty-six hours' digestion.

(2) Unless great care is used, the addition of lead acetate to the hot alcohol solution will cause violent ebullition and partial loss of contents. A safety tube helps to overcome this difficulty.

(3) In determining the lead content of the caffetannate it is advisable to filter the caffetannate through a tared gooch. This will allow of digestion of contents in nitric acid and precipitation of the lead with sulphuric acid without using a filter paper, the carbon of which does not completely oxidize and produces a blackening of the lead sulphate. The final weighing of the sulphate should also be made in a gooch.

*Caffein method.*—It is suggested that the caffein iodine precipitate does not form immediately and that the low results are due to filtering and titrating the solution too quickly. Other work indicates that after the iodine is added the flask should be allowed to stand in an ice chest overnight before titrating.

Much better results can be secured by Gomberg's original method for caffein, as given in the *Journal of the American Chemical Society* (1896, 18: 331), and modified as follows:

Extract 2 grams some time with four portions of water, cool, and make to 1,000 cc. Treat 500 cc with 15 cc of saturated lead acetate solution, let settle, filter, remove lead with hydrogen sulphid, boil off excess of hydrogen sulphid, divide filtrate into two parts, concentrate each to 50 cc, add 0.2 cc of concentrated hydrochloric acid to one and 0.5 cc of acetic acid to the other, cool to 15° C., add 20 cc of tenth-normal iodine solution, stopper flask, and let stand in ice two hours, filter on a gooch. Caffein does not precipitate unless mineral acid is present, so the acetic acid portion shows if any other materials are present which would precipitate with the iodine solution. If any absorption of iodine is found in the acetic portion, it must be deducted from the titration containing mineral acid. The difference represents the iodine used up in the formation of the periodid of caffein: 1 cc of tenth-normal iodine equals 0.00485 gram of caffein. Using this method, 0.78 per cent of caffein was obtained from the coffee reported.

*Krauch method for extract in tea.*—The bulk of sample (20 grams) makes complete removal of water-soluble substances almost impossible. The absorption of water by large filter paper and on surface of flask during weighing is also a serious objection to the method. If sample is ground, filter paper is clogged and filtration prevented.

*Doolittle and Woodruff method.*—Care should be used to keep the entire sample in the boiling liquid during extraction or low results will be obtained. Any loss of water by evaporation should be replaced.

#### NOTES BY REFEREE.

The discrepancy in the results obtained by the two analysts with the Trillich and Gökkel method is due principally to the fact that Mr. Woodruff used 5 cc of basic lead acetate in the precipitation instead of 1 cc, as prescribed in the method. Determinations made by the referee on the same sample gave 10.08 per cent where 2 cc of basic lead acetate was used and 12.04 per cent when 4 cc was used. The lower results obtained by Mr. Woodruff in the caffein estimation may have been due to the greater volume of solution in which the caffein periodid was precipitated, he using a volume of 100 to 150 cc, while the referee employed a volume of 20 cc. Experiments made by Mr. W. C. Taylor in the writer's laboratory have shown the necessity for concentrating the caffein solution to small bulk.

The determinations made of extract in tea by the referee convinced him of the great superiority of the Doolittle and Woodruff modification over the Krauch method as regards convenience, time, and liability to error.

#### COCOA PRODUCTS.

The following results were obtained from the collaborating chemists on cocoa products:

##### *Cooperative work on cocoa products.*

Analyst.	Plain chocolate (Sample A).		Milk chocolate (Sample B).	
	Crude fiber.	Crude starch.	Lactose.	Sucrose.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
G. M. Bartlett, Boston.....	2.25	11.61	5.05	25.72
R. W. Hilts, Philadelphia.....	2.50	9.69	4.83	27.83
	2.65	9.97	5.25	27.32
A. G. Woodman, Boston.....	2.84	10.37	5.13	27.02
	2.69	10.03		

*G. M. Bartlett:* The conversion of the starch was carried out as outlined, no difficulty occurring in the procedure. The aliquot for precipitation was obtained as follows: After converting, neutralizing, and adding basic lead acetate the sample was made up to volume at about 35° C. To 100 cc at this temperature was added the 60 per cent of sulphuric acid, cooled so that the volume of liquid contracted to 100 cc. It was necessary to cool only to about 18° C. The sample for precipitation was taken when the liquid had contracted to the mark. Two determinations were made—one by precipitating by the Walker-Munson method (J. Amer. Chem. Soc., June, 1906,) and the other following the method in Bulletin 107. The latter gave 11.83 per cent of starch.

In determining crude fiber the electric stove was used for boiling the 1.25 per cent sulphuric acid and caustic soda. There was but little frothing. The filter paper and crude fiber were dried at 100° C and over sulphuric acid. During weighing the filter paper gained in weight. I do not care for this method of getting the weight of the crude fiber, even though it is not to be ignited, and would prefer filtering on a weighed platinum gooch filter.

In calculating the sugar in chocolate by Dubois's method *a* it seems illogical to multiply  $(a-b)$  by  $1.05x$  ( $x$  equaling the volume obtained by dissolving sugar in 100 cc of water) rather than by 105 plus the increase in volume due to the solution of the sugar. This actually makes but little difference in the result, but the following statement of the formula seems preferable:

$$\frac{(a-b)(105+x')}{144-\frac{t}{2}} = \text{per cent sucrose.}$$

Where  $x'$  = increase of volume owing to the solution of the sugar in water. In calculating the lactose the complete formula reads: Per cent lactose =  $C \times 4 \times 1.11 \times 1.05x \times 1.264$ , where  $x$  = volume of solution when the sugar is dissolved in 100 cc.

*R. W. Hiltz:* The samples on arrival were immediately placed in glass-stoppered bottles. Before removing portions for analysis they were rubbed down to a coarse powder in a large porcelain mortar and mixed as well as possible. This was done quite rapidly, both to avoid possible changes in moisture content and to avoid formation of a pasty mass.

*Crude fiber:* First filtration was made on closely woven linen in a 4-inch Büchner funnel with light suction. Second filtration was on a 11 cm B & A ashless filter paper without suction. Both filtrations were rapid and satisfactory.

*Starch:* Results are multiplied by the factor 1.01 to correct for the dilution of 100 cc of the solution by the 1 cc of sulphuric acid.

*Sugars:* The method of Dubois was followed exactly. It was necessary in extracting with water to break up with a glass rod the compact cake left after centrifuging the last time with gasoline. Inversions were made in the cold (50 cc + 5 cc of hydrochloric acid, being allowed to stand over night. All volumes were adjusted at 20° and all polarizations were made in jacketed tube at exact temperatures. The actual polariscope readings (averages of four to five close readings) illustrate the very great influence that small differences in readings have upon the results, in these dilute solutions. In spite of this fact, the method seems to be satisfactory and convenient for judging milk chocolates. The methods are, in my opinion, in as simple a form as possible, and can not well be improved.

#### RECOMMENDATIONS.

In view of the small number of collaborators it is hardly possible for the referee to make any formal recommendations based on collaborative work. It is evident, however, that the study of certain of these methods should be continued by the association, especially the caffeine estimation and the determination of sugars in chocolate. There would appear to be no reason why the determination of extract in tea as outlined by Doolittle and Woodruff should not be substituted for the cumbersome Krauch method. The experience of the referee on numerous samples of cocoa products suggests that the requirement for filtering and weighing the crude fiber on a

<sup>a</sup> J. Amer. Chem. Soc., 1907, 29: 556; see also Bul. 107, Rev., p. 256.

paper should be omitted, as the determination can be made more conveniently on a gooch crucible as ordinarily used.

Attention is also called to the accompanying paper involving some of Mr. W. C. Taylor's work on caffetannic acid and caffenin.

### ESTIMATION OF CAFFETANNIC ACID AND CAFFEIN IN COFFEE.

By A. G. WOODMAN and W. C. TAYLOR.

In connection with an examination of the methods for coffee analysis the writers have made a study, in the limited time available, of the provisional methods for determining caffetannic acid and caffenin, especially the former.

#### CAFFETANNIC ACID.

Experience has shown that with the directions as given at present it is practically impossible to obtain concordant results or a lead caffetannate of constant composition.

It has been the general experience of those who have worked with the Krug method that it is tedious in the extreme, and, furthermore, that the composition of the so-called lead caffetannate obtained varies with the conditions of precipitation. It was our purpose to ascertain if possible the source of some of these difficulties.

It was seen early in the work that variations in the amount of lead acetate used for precipitation gave variations in the proportion of lead caffetannate obtained, as well as in its content of lead. This is shown in the following table, in which the determinations were made on aliquot portions of a coffee infusion and varying amounts of saturated lead acetate were used, all other conditions being kept constant.

*Determination of caffetannic acid, using varying amounts of lead acetate.*

Lead acetate.	Caffetan- nic acid by Krug's factor.	Lead in precipitate.
cc.	<i>Per cent.</i>	<i>Per cent.</i>
1	7.66	50.04
2	9.73	50.35
4	11.14	-----
6	11.70	48.31
8	12.28	55.16
10	14.28	55.93

The averages of several results are stated in each case, although the results showed very considerable variation. While too much reliance can not be placed on these figures, owing to variations among themselves, they show the necessity of using a definite amount of lead acetate for the precipitation.

Another source of error is the difficulty of washing the lead caffetannate free from lead acetate. Those who have attempted it know the tediousness and the difficulty of washing the precipitate on the filter. It is of course necessary to use alcohol of 90 per cent strength in washing, on account of the solubility of the precipitate in water or dilute alcohol. On the other hand, the lead acetate which is to be removed is only slightly soluble in 90 per cent alcohol. Hence it will be readily seen that it is practically impossible to wash the bulky precipitate on the filter. It is true, also, that when the wash water no longer reacts for lead the precipitate is not necessarily free from it, since owing to its character the wash water easily forms channels and does not wash it thoroughly.

After numerous experiments, washing by the centrifugal machine was tried, giving several treatments with 90 per cent alcohol in the tubes of the centrifugal before trans-



ferring to filter paper. This method gave results which were in much closer agreement, as shown by the following results on the same sample:

Per cent caffetannic acid.....	9.69	9.69	9.57	9.40
Per cent lead.....	48.28	48.35	48.01	48.71

Tests made on a considerable quantity of the lead caffetannate washed in this way showed it to be free from fat and nitrogen.

It would seem as if the long process of digestion with water and with alcohol prescribed by Krug could be materially shortened. In much of our work extracts of the coffee were prepared by the use of a shaking machine, shaking the sample for an hour with water and half an hour with alcohol. Results obtained in this way agree very well with those obtained by the official method of digestion, although there is evidence to show that neither method extracts all of the caffetannic acid.

Regarding the vexed question of the composition of caffetannic acid, we seem not much nearer a settlement. The views previously held, which seem to lead to the formula for a di-glucosid, have been clearly set forth in Bulletin 105 by Mr. Howard. Lack of time prevented any extended investigation of this problem, but an endeavor was made to confirm the work of Cazeneuve and Haddon in regard to the di-glucosid formula for caffetannic acid. We were unsuccessful, however, in preparing more than traces of the osazone prepared by them, although carrying out the experiments exactly in the manner prescribed. In this connection the paper recently published by Görter<sup>a</sup> is of interest, in which the correctness of the Cazeneuve and Haddon formula is questioned. Görter states that he was unable to form more than a few small crystals of the osazone, which he was unable to isolate and considered that it was due to some impurity in the caffetannic acid. The caffetannic acid is considered by Görter to be a mixture of chlorogenic and coffalic acids. Numerous derivatives and salts of these acids have been prepared and are described by the author to support his contention.

The method for carrying out the Krug test which we found to work most satisfactorily may be summed up as follows:

To 2 grams finely ground coffee (passing 0.5 mm sieve), add 10 cc of water and shake for an hour in a mechanical shaking device. Add 25 cc of 90 per cent alcohol and shake again for half an hour. Filter and wash with 90 per cent alcohol. Bring the united filtrate and washings, about 50 cc, to boiling and add 6 cc of saturated lead acetate solution. Separate the precipitated lead caffetannate by means of a centrifuge, decanting the supernatant liquid through a tared filter. Repeat the centrifugal treatment twice with 90 per cent alcohol, decanting each time through the filter. Transfer the precipitate to the filter and wash free from lead. Wash with ether, dry at 100°, and weigh. The weight of precipitate multiplied by 0.51597 gives the weight of caffetannic acid.

#### CAFFEIN.

In the work on caffein a comparison was made of three methods: The official method (Bul. 107, p. 154); the titration of caffein with iodine, according to Gomberg, in the filtrate from the lead caffetannate; and the method proposed by Görter in the paper previously mentioned.

Our attention has been directed by Mr. C. D. Howard to a source of error in the provisional method, arising from the fact that the extraction with dry chloroform of the sand-magnesia mixture does not yield the whole of the caffein. Mr. Howard says in his letter:

My practice has been to add to the concentrated filtrate, contained in a tin-foil dish, about 10 grams of sand and 1 gram of magnesium oxid, evaporate and dry in the water oven for a short time. The brittle mass, easily stripped from the dish, I grind finely, place in a paper extraction cartridge, and extract for ten to twelve hours in the usual way.

<sup>a</sup> Annalen, 1908, 359: 217.

Now, my recent experience has been that if the extracted residue be shaken with water and the latter further extracted, an additional quantity of caffeine, sometimes equivalent to 10 per cent of the whole, is thus obtained.

Our experience has been a similar one. To illustrate by a specific instance, the residue from twenty hours' extraction with chloroform was shaken with water, filtered, and the aqueous solution extracted four times with chloroform. One-half the chloroform extract was tested for caffeine, and gave positive tests with Wagner's reagent and by the "murexid" test. The other portion showed by a Kjeldahl determination 0.0035 gram of caffeine, corresponding on the whole sample to about 10 per cent of the amount present.

Görter finds that a considerable proportion of the caffeine in coffee is present as a double salt, the potassium caffeine chlorogenate, from which the caffeine is extracted by dry chloroform only with great difficulty. Whether or not this be the cause of the incomplete extraction, it is evident that the official method needs revision.

Preliminary experiments with the Gomberg method showed it to be practicable for the small amount of caffeine (approximately 20 mg) that would be present in the filtrate from the lead caffetannate, providing the volume of solution were not over 25 cc. On account of the slight solubility of the caffeine periodid in the wash water it was found best in working with this small amount to suck the precipitate as dry as possible on the gooch filter and not to wash it. Determinations made on 20 mg of caffeine in 25 cc of water in this way gave from 98 to 99 per cent of the caffeine present. Numerous experiments made on the filtrate from the lead caffetannate precipitate by precipitating the lead with hydrogen sulphid and evaporating the filtrate gave fairly concordant results, which were uniformly lower than those given on the same coffee by the other methods for caffeine. It was observed that the variations in amount of caffeine as determined in this way corresponded roughly with the variations in amount of caffetannic acid as found by the Krug method. Whether these variations and low results are due to incomplete extraction of caffeine by the process of digestion employed in the Krug method is a matter which we expect to investigate further.

Görter's method was not given a thorough trial. As far as the work goes it has been satisfactory, and the method is worthy of further trial by the association. It reads briefly as follows:

Eleven grams of the finely powdered coffee are moistened with 3 cc of water and after standing a half hour extracted for three hours in a Soxhlet extractor with chloroform. The extract is evaporated, the residue of fat and caffeine treated with hot water, filtered through a cotton plug, and washed with hot water. The filtrate and washings are made up to 55 cc, 50 cc pipetted off and extracted four times with chloroform. This chloroform extract is evaporated in a tared flask and the caffeine dried at 100° and weighed.

In the determinations made it has never been possible to weigh the caffeine directly on account of impurities, the caffeine having been calculated from a determination of nitrogen in each case. From the work done there seems to be a strong probability that a combination of the Gomberg and Görter methods will prove to be the best and most convenient process for determining caffeine in coffee.

## SECOND DAY.

### FRIDAY—MORNING SESSION.

#### REPORT ON THE DETERMINATION OF NITROGEN.

By CHARLES I. PENNY, *Referee.*

An apology is due the association for failure to carry out the instructions of 1906 concerning the permanganate methods. Another phase of the nitrogen question considered last year and discussed in correspondence with the National Fertilizer Association seemed to outweigh in importance and urgency all others, namely, the determination of total nitrogen in mixed fertilizers to which nitrate of soda is added. With a view to making a thorough investigation of this subject, the following instructions were sent to such members of this association as were supposed to be interested in the work and to more than a score of analysts named by the secretary of the National Fertilizer Association:

#### INSTRUCTIONS.

Chemists cooperating in this work are requested to give their attention exclusively to methods applicable in the presence of nitrates, Bulletin 107, page 7 (c), page 8 (d), page 10 (g) and (h). There seems to be urgent need of this investigation, especially since the present methods used to determine nitrate nitrogen have been formally called in question by the representatives of great commercial interests.

No samples are sent herewith, as it is thought that each analyst, by calculating the nitrate used from a solution of nitric acid carefully compared with his own standard alkali and acid, may get more reliable results, through the balancing of possible errors, than from using a common substance.

Let the source of nitrate used be a solution of pure nitric acid, about fifth normal, most accurately titrated against the standard alkali used in the Kjeldahl work. In each case measure accurately into the digestion flask enough of this nitric acid to contain 30 to 50 milligrams of nitrogen.

(1) Follow method (c), disregarding the water in the nitric acid and without neutralizing.

(2) Follow likewise method (d).

(3) Follow method (g), adjusting the proper amount of water, distilling first with magnesia, then with caustic soda and water added to the residue in the distillation flask, collecting *separate* distillates and titrating each *separately*.

(4) Similarly follow method (h), beginning in second line "in a distillation flask," etc.

If the yield of nitrogen is less than the calculated in (1), (2), (3), or (4), test the residue in distillation flask for nitrates.

(5) Proceed as in (1), (2), (3), (4) with the preliminary addition of 2 grams of cane sugar to the digestion flask.

(6) Proceed as in (1), (2), (3), (4) with the preliminary addition of 1 gram, accurately weighed, of organic nitrogen substance, such as dried blood, fish scrap, or tankage, to the digestion flask. In a separate operation treat 1 gram, accurately weighed, of this added substance similarly except that no nitrate be present; that is, analyze the added substance alone according to (c), (d), (g), and (h). Any nitrate-free mixed fertilizer may be used for the added substance.

While the above plan entails much work, it is hoped that a large number of chemists will test at least some of the several official methods in question, if not all. The figures for each separate determination should be reported and the precise method pursued should be fully explained. The results of any other plan of studying the nitrate question, carried out by chemists at their own suggestion, will be welcomed.

Answers were received from five chemists engaged in official work and three engaged in commercial work, viz, Messrs. E. M. Bailey, New Haven, Conn.; F. B. Carpenter, Richmond, Va., reporting work of Mr. W. D. Cooke; H. S. Lansdale, Buffalo, N. Y.; C. B. Morrison, New Haven, Conn.; J. Bernard Robb, Richmond, Va.; B. F. Robertson, Clemson College, S. C.; Paul Rudnick, Chicago, Ill., and T. C. Trescot, Washington, D. C.

It is regretted that cooperation has not been more general, but the work required seemed burdensome, and doubtless few could find the time to engage in it. Several of the cooperating chemists, however, have shown extraordinary industry, reporting an amount of work seldom equaled in voluntary investigation of this sort. The questions involved in the plan of work are not less than 14; hence analytical results are too complicated to admit of convenient tabulation. It seems better, therefore, to deduce from the analytical figures the answers to the several questions.

Before judging the results it is well to bear in mind the reasonable expectation of agreement or accuracy from a number of chemists working on the same subject. Last year on the simpler problem of determining nitrate-free nitrogen, the work of over 50 chemists, possibly the largest number of the association ever engaged on a single question at one time, seems to indicate that about 98 per cent of the truth is the average with present methods and present personnel. Then in the more difficult question of nitrates and the separation of several forms of nitrogen, this expectation would seem to be at least high enough. Thus methods for nitrates that give as much as 98 per cent of theory are at least as accurate as the average results on nitrate-free substance. While this limit may easily be exceeded by experienced and skillful individual analysts, it is useless to deny that it is not exceeded by the average results.

The analysts used chiefly as their source of nitrate nitrogen amounts of their own standardized nitric acid containing from 28 to 160 mgs of nitrogen. The results obtained are reported as percentages, the basis of which is the amount of nitrogen that should have been obtained.

The questions involved follow, with the answers deduced from the figures of the several analysts.

*Percentages of nitrogen recorded based on amount present, using different methods.*

*[Is nitric acid, in the absence of organic matter, reduced to ammonia without loss?]*

(1) BY METHOD (C).

Analyst.	Number of determinations.	Range of determinations.	Average.
		<i>Per cent.</i>	<i>Per cent.</i>
Bailey.....	2	74.5-82.9	78.7
Cooke.....	5	94.0-96.0	95.0
Lansdale.....	2	97.4-98.6	98.0
Morrison.....	2	86.6-86.7	86.7
Robb.....	8	87.0-96.0	91.4
Robertson.....	4	-----	99.2
Rudnick <sup>a</sup> (Thio.).....	4	61.1-76.9	67.4
(Zn).....	4	80.8-86.8	83.2
(Zn+Thio.).....	4	76.4-82.5	79.8

(2) BY METHOD (D).

Bailey.....	2	74.8- 75.7	75.3
Cooke.....	3	96.0- 96.0	96.0
Lansdale.....	2	95.7- 97.4	96.6
Morrison.....	1	-----	76.4
Robb.....	3	93.0- 95.0	94.0
Robertson.....	4	-----	98.5
Rudnick.....	8	55.0-100.0	76.0

<sup>a</sup> Analytical work reported by F. W. Rudnick throughout the report was done by F. Fenger, K. J. Monrad, and A. C. Johnson.

Percentages of nitrogen recorded based on amount present, using different methods—Cont'd.

## (3) BY METHOD (G).

[Distilled off magnesia followed by soda, the sum of both distillates being used.]

Analyst.	Number of determinations.	Range of determinations.	Average.
		Per cent.	Per cent.
Bailey.....	2	99.8-100.0	99.9
Cooke.....	2	94.0-95.0	94.5
Morrison.....	2	98.1-98.9	98.5
Robb.....	6	89.0-98.0	93.5
Rudnick.....	8	54.9-95.0	76.8

## (4) BY METHOD (H).

Bailey.....	2	95.6-99.3	97.5
Morrison.....	2	95.4-97.2	96.3
Rudnick.....	12	91.6-99.9	95.8

[Is nitric acid reduced to ammonia without loss in the presence of 1.4 to 2 grams of sugar?]

## (5) BY METHOD (C).

Bailey.....	2	35.6-49.5	42.6
Morrison.....	2	39.6-63.9	51.8
Robb.....	3	61.0-63.0	61.7
Robertson.....	4	.....	100.0
Rudnick.....	8	71.7-89.9	79.4
Robertson <sup>a</sup> .....	4	.....	98.0

## (6) BY METHOD (D).

Bailey.....	2	40.0-42.1	41.1
Morrison.....	2	24.8-44.2	34.5
Robb.....	3	61.0-63.0	61.7
Robertson.....	4	.....	99.5
Rudnick.....	8	70.0-86.2	76.7
Robertson <sup>a</sup> .....	4	.....	99.0

## (7) BY METHOD (G).

[Distilled off magnesia followed by soda, the sum of both distillates being used.]

Bailey.....	2	95.4-98.9	97.2
Morrison.....	2	98.0-99.3	98.7
Rudnick.....	3	103.6-111.2	<sup>b</sup> 106.6

## (8) BY METHOD (H).

Bailey.....	2	97.0-110.0	103.5
Morrison.....	2	95.8-98.1	97.0

[Is the sum of nitrogen in nitric acid and nitrogenous organic matter fully recovered as ammonia?]

## (9) BY METHOD (C).

Bailey.....	2	79.2-86.5	82.9
Morrison.....	2	84.0-87.1	85.6
Rudnick (Thio.).....	3	77.4-82.4	80.7
(Zn+Thio.).....	4	66.6-74.7	70.6

<sup>a</sup> In the presence of 1 gram of a nitrate-free mixed fertilizer instead of sugar.

<sup>b</sup> In these determinations by the Ulsch method, the small quantity of water undoubtedly caused spurting of alkali sufficient to drive it over into the condenser. The bumping was terrific. Rudnick.

Percentages of nitrogen recorded based on amount present, using different methods—Cont'd.

## (10) BY METHOD (D).

Analyst.	Number of determinations.	Range of determinations.	Average.
Bailey.....	2	<i>Per cent.</i> 77.5-81.0	<i>Per cent.</i> 79.3
Morrison.....	2	77.3-86.5	81.9
Rudnick.....	8	42.6-43.7	55.0

[Is any of the nitrogen in nitrate-free and ammonia-free nitrogenous bodies obtained?]

(11) BY METHOD (G).<sup>a</sup>

Bailey.....	2	1.9-2.4	2.2
Morrison.....	2	2.4-3.5	3.0

<sup>a</sup> These figures were obtained distilling with magnesia only. Soda being added and distillation continued, the following additional percentages were obtained: Bailey, 8.3 to 9.3, average 8.8; Morrison, 8.3 to 11, average 9.7. These four determinations were made on cotton-seed meal containing 0.0739 gram of nitrogen, on which nitrogen the percentages are based.

## (12) BY METHOD (F).

Bailey and Morrison, who alone worked on this question, report that distillation was rendered impossible by excessive frothing.

[How complete is the liberation of ammonia when distilled with magnesia?]

(13) BY METHOD (D).<sup>a</sup>

Analyst.	Number of determinations.	Range of determinations.	Average.
Bailey.....	4	<i>Per cent.</i> 92.2-97.9	<i>Per cent.</i> 94.3
Cooke.....	2	19.0-22.3	20.7
Morrison.....	4	93.6-97.9	95.8
Robb.....	6	15.5-31.6	22.4
Rudnick.....	8	42.4-73.7	63.8

<sup>a</sup> These figures represent the fraction of total ammonia liberated by magnesia, the complement lacking to make 100 per cent having been liberated by soda. Thus, Cooke's lowest result was 19 per cent distilled from magnesia and the remaining 18 per cent was obtained by adding soda solution and continuing the distillation. Trescot, reported below, recovered practically all of the ammonia by magnesia alone.

(14) *Is the loss of nitrogen by methods (c) and (d) caused by heat generated in mixing the acid and water?*

Rudnick, using potassium nitrate instead of a solution of nitric acid, obtained the following figures, and as compared with these results those found with the solution of nitric acid by the same methods averaged 79.8 to 66.1 per cent. Trescot obtained 80 per cent when using nitric acid, as compared with 100 per cent with potassium nitrate.

*Rudnick's results, using potassium nitrate.*

Method.	Number of determinations.	Range of determinations.	Average.
(c) (Zn+Thio.)...	4	<i>Per cent.</i> 93.5-97.4	<i>Per cent.</i> 95.4
(d).....	4	88.3-94.0	92.1

The work of Trescot on potassium nitrate is reported separately, as follows:

## (15) BY METHOD (D).

Material.	Number of determinations.	Range of determinations.	Average.
Potassium nitrate.....	4	<i>Per cent.</i> 99.8-100.1	<i>Per cent.</i> 100.0
Nitric acid, neutralized and evaporated.....	5	97.7-99.2	98.8
Potassium nitrate and dried blood.....	3	98.0-98.8	98.5
Potassium nitrate and ground bone.....	3	100.6-101.6	101.1

## (16) BY METHOD (G). (ULSCH-STREET.)

Potassium nitrate.....	3	99.8-99.8	99.8
Potassium nitrate and dried blood.....	2	99.2-99.2	99.2
Potassium nitrate and ground bone.....	2	99.2-99.2	99.2

Impurities in reagents, reckoned on the nitrogen as the basis of percentage, were reported as follows by two analysts only:

*Percentage of impurities in reagents based on nitrogen present.*

Analyst.	Method.			
	(c)	(d)	(g)	(h)
Bailey.....	0.9	0.7	0.4	4.4
Morrison.....	.8	.7	.4	4.4

## COMMENT BY ANALYSTS.

Carpenter, reporting the work of Cooke, says:

The Kjeldahl method, or in fact any of the methods described in the "Official Methods of Analysis," are very unsatisfactory for the determination of nitrates. In our own work for the analysis of nitrate of soda, nitrate of potash, etc., we use an electrolytic method.

Robb says:

I noticed a very perceptible loss of nitric oxid when I added the salicylic-acid mixture both in the case of the nitric-acid solutions containing a nitrate-free fertilizer and those containing cane sugar.

Rudnick says:

Inasmuch as all of the work could not be carried out in full, such features as seemed to me of less interest from the standpoint of a fertilizer manufacturer's laboratory were omitted. These had to do chiefly with the determination of nitrate nitrogen per se. We are not so much interested in this feature, inasmuch as there are several good methods now in use for this purpose. Of these we prefer the Schloesing-Wagner method, as described in Bulletin 107 of the Bureau of Chemistry, page 111.

It is not the determination of nitrate nitrogen that we are so vitally interested in but the determination of total nitrogen where nitrates are present.

The results with the fifth-normal nitric acid were very unsatisfactory indeed. This was probably due to the great dilution with water, which we never meet with in our work. Every precaution was taken to prevent undue heating on adding the strong salicyl-sulphonic acid.

A great many more determinations than appear in the preceding report were made in this laboratory, but none of them was any more satisfactory than those reported on, and there seemed to be no special significance in the results obtained. The Ulsch method as given in Bulletin 107 with a number of variations has been tried in this laboratory at various times during the past years, but has never given satisfactory

results. The zinc-iron method works very smoothly, and some of the results are quite as satisfactory as those obtained by the Schloesing-Wagner method.

But, as stated above, we are mostly interested in the Gunning and Kjeldahl methods for total nitrogen in the presence of nitrates. These methods have not given satisfactory results in the past. There are some details in the manipulation of these methods not described in Bulletin 107 which seem to tend materially to a closer approximation of the true amount of nitrate nitrogen, such as quick covering of the sample with enough salicyl-sulphonic acid to absorb all of the evolved nitrogen oxides, sufficient time for thorough nitration of the acid, thorough cooling of the mixture before adding the thiosulphate, and heating to a free boil before adding the mercuric oxid or the potassium sulphate, respectively. We find also that the tendency to foam is very largely obviated by heating the flask containing the mixture in a boiling water bath for an hour, or possibly less, prior to adding the thiosulphate.

In view of the poor results obtained with nitric acid it seemed that the use of a pure nitrate weighed directly into the digestion flask might prove much more satisfactory for determining the accuracy of the various methods. Our work following this plan has given much better results than by using nitric acid as the source of the nitrate.

In the large majority of cases we have to do with mixed fertilizers containing a nitrate. Any inaccuracies due to the water present in nitric acid or in the solution of a nitrate could easily be obviated by evaporating to dryness in the digestion flask, preferably in vacuo, after neutralizing when necessary to prevent the loss of nitric acid.

But even in the determination of an added nitrate the results leave much to be desired. This is not only the case in this particular work, but has been a generally recognized fact among fertilizer chemists for a long time. If the secret of success lies in certain details of manipulation not commonly known, which make the method reliable, these details should certainly be mentioned in the description of the method.

Trescot says:

I inclose results on neutralizing with soda and evaporating down, also on mixtures of potassium nitrate and blood, and potassium nitrate and bone by the Gunning modified method for nitrates and the Ulsch-Street method. These results only confirm my previous work on such materials with these methods. The zinc-iron method is a failure in my hands. I never could get concordant results. If there is anything more I can do, let me know and I will be glad to do it; only I do not wish to repeat my work on the Ulsch-Street and Gunning modified methods, for after the most careful checking for many years I am convinced that if handled properly, and on dry materials, both methods will give all the nitrate present.

#### DISCUSSION OF RESULTS.

The opinions quoted and results reported show wide variations. By methods (c) or (d) with and without organic matter, Robertson and Trescot alone get satisfactory results; those of the other chemists are low, mostly impossible, in fact. This may be due to heat generated by the acid and water, as many chemists think and as Rudnick's and Trescot's work on potassium nitrate seems to show. On the other hand, Robertson's work shows no appreciable loss. An important question is, Must not this possible loss from heat be reckoned with even with a comparatively dry substance? Even with potassium nitrate Rudnick falls far short of acceptable accuracy. In view of these facts may not a too speedy application of heat in methods (c) and (d) cause loss of nitrate vapors? It is a practice of some careful analysts to allow nitrate samples to stand several hours after the thiosulphate or zinc dust is added. Hence, recommendations are offered fixing a minimum time limit in methods (c) and (d).

Method (g) as carried out by Bailey, Morrison, and Trescot gives good results; as carried out by the other analysts uncertain and varying results, mostly far too low. It must be noted, however, that to test the completeness of the reduction of nitric acid to ammonia the distillation is made at first off magnesia and completed off soda, except in Trescot's work, the amount of ammonia from each distillation being estimated separately and the sum taken. It appears further from this process that the distillation from magnesia is usually far from complete, giving in several instances less than one-fifth of the total amount of ammonia; that is, less than one-fifth of what



should have been obtained. As method (g) permits the use of magnesia only, no soda, in distilling, it is evident how very inaccurately this method is practiced by some chemists. It would seem that the magnesia distillation process is conducted by many chemists less successfully than any other analytical process.

Furthermore, the work of Bailey and Morrison shows that by method (g) some ammonia is obtained from cotton-seed meal, about 2 or 3 per cent of the total nitrogen in the substance coming off as ammonia, when the distillation is off magnesia, and about three times as much in addition, when soda is used in the distillation. This is fortunately a compensating error and contributes something toward making up the deficit just described due to distilling ammonia off magnesia.

As practiced by some chemists the process of distilling off magnesia is worse than useless. As method (g) does not affect the amount of total nitrogen, errors are not so serious, however, as in methods (c) and (d).

Method (h) shows better agreement among different chemists and also gives figures somewhat approximating theory.

The only report on method (f) is to the effect that excessive frothing prevented distillation.

Of the 54 answers to the 14 questions stated above, 39, or nearly three-fourths, must be considered unfavorable, either as failing to reach the 98 per cent standard of accuracy or as showing reactions contrary to the plan and purpose of the method.

It must be admitted, whatever may be the inherent accuracy of the several methods here discussed, that most of them in the hands of some experienced chemists fail to give reasonably reliable and accurate results. It must also be admitted that these methods with more or less variation in detail are commonly accepted by chemists as reliable, and, as this report shows, may be made to give exceptionally accurate results. Obviously methods so firmly established, and by some analysts so successfully employed, are hardly to be condemned, or even seriously questioned, without a study of them by a large number of chemists. The data obtainable for this report are too meager to justify any criticism or proposed radical change.

The referee is still of the opinion that the methods here discussed are fairly accurate when properly followed, less accurate perhaps in the hands of some analysts than methods applied to simpler determinations, such as of nitrate-free nitrogen, but yet as accurate as the difficulty of the case permits; and furthermore, that unquestionably these methods are not always successfully followed, as evidenced by the criticisms of chemists as well as by the reported results, and that probably some analyses made according to these methods are erroneous, giving too low results; that the complaint of the officers of the National Fertilizer Association may possibly be based on fact in certain cases, probably due in part to erroneous analyses and in part to actual loss of nitric acid, but that it is not within the power of this association at present to remedy the evil complained of, if it exists.

#### RECOMMENDATIONS.

Two recommendations of 1907, referred to the referee for 1908, are recommended for adoption as official. (Nos. 2 and 4, Circular 38, page 1, or Bulletin 116, page 129.) These changes relate to the use of copper sulphate in the Kjeldahl and Gunning methods. For detailed statement of changes see page 183.

Recommendation 3: Bulletin 107 Rev., page 8, fourth line from top, after the word "time" insert: "Allow the flask to stand without heat for not less than six hours."

Recommendation 4: Same reference, page 8, under (d) (3) "determination," fifth line of paragraph, after word "and" insert: "Allow the flask to stand without heat for not less than six hours; then". So changed the sentence beginning with "Add 5 grams" would read: "Add 5 grams of sodium thiosulphate and allow the flask to stand without heat for not less than six hours; then heat the solution for five minutes; cool; add 10 grams," etc.

At the close of the reading of the nitrogen report, the president announced the following committees:

Committee on amendments to the constitution: J. P. Street, J. T. Willard, P. F. Trowbridge.

Committee on nominations: R. J. Davidson, C. H. Jones, B. B. Ross.

Committee on resolutions: L. I. Van Slyke, A. J. Patten, V. K. Chestnut.

## REPORT ON INORGANIC PLANT CONSTITUENTS.

By H. D. HASKINS, *Referee*.

The work on inorganic plant constituents has been along lines recommended by the referee of the preceding year, particularly with reference to the development of a method for the determination of iron and aluminum in ash. The sample which has served for the work was prepared by thoroughly mixing the ash of a species of *Lycopodium*, known to contain a large proportion of aluminum, with a finely ground and incinerated sample of wood ashes, the latter being known to contain considerable quantities of iron.

### PROPOSED METHODS.

The method proposed for study contains some of the features incorporated in the official method of determining ferric and aluminic oxids and phosphates in soils (Bul. 107, p. 15). See also Bul. 56, Proceedings of the Fifteenth Annual Convention of the association in which recommendations are made by Hartwell in regard to a method described in Crooke's Select Methods of Chemical Analysis. The method as outlined for the work this year was in detail as follows, a hydrochloric acid solution of the ash being used:

#### SEPARATION OF FERRIC AND ALUMINIC OXIDS IN ASH ANALYSIS.

Use a solution corresponding to 0.2 gram of ash. After removing the phosphoric acid the filtrate from the precipitate of ammonium phosphomolybdate, consisting of a nitric acid solution of molybdic acid, ferric oxid, alumina, lime, and magnesia, is placed in a beaker and cautiously neutralized with ammonia, care being taken that the temperature does not rise above 40° C. and that the alkali is added only in slight excess; allow to stand in a warm place until the precipitate completely settles, filter the clear supernatant fluid, wash the precipitate with hot water by decantation, then transfer it to the filter, and finish the washing. Next, redissolve the precipitate through the filter in weak, hot nitric acid (1 to 5), reprecipitate with ammonia, filter, and wash in the same careful manner. The precipitate is dried, ignited, and weighed as ferric oxid and alumina.

METHOD (b).—The weighed precipitate of ferric oxid and alumina is dissolved, on the hot water bath, in a covered flask by the addition of about 20 cc of dilute sulphuric acid (1 part sulphuric acid to 4 parts water). The iron is reduced to the ferrous state by adding iron-free metallic zinc (about 5 decigrams at each addition) until the solution is completely decolorized and the iron is all reduced; cool by immersing in cold water, dilute with cold distilled water which has been recently boiled, pour off and wash into beaker, leaving behind any residue of zinc. Titrate with standard permanganate solution.

METHOD (c).—An aliquot part of the original solution A, corresponding to 0.2 gram of ash, is evaporated on hot water bath with the addition of 10 cc of sulphuric acid until all hydrochloric acid is expelled; dilute with water, reduce with zinc, and estimate iron by standard solution of potassium permanganate. The per cent of ferric oxid obtained is deducted from the per cent of ferric oxid and alumina, corrections being made for filter ash, to obtain the per cent of alumina.

## MAKING AND STANDARDIZING PERMANGANATE SOLUTION.

Dissolve 2.82 grams of pure crystallized potassium permanganate in distilled water by the aid of heat; cool and dilute to 1 liter and preserve in stoppered flask. Standardize this solution by titration with metallic iron solution as directed in the second American edition of Fresenius's Quantitative Chemical Analysis, pages 268-269.

A copy of the method as outlined above, together with the ash sample, was sent to eight chemists who had signified their intention of cooperating in this work. The results received from five analysts are given in the tabulated statement which follows:

*Cooperative work on ash sample.*

Analyst.	Method B.		Method C.	
	Alumina (Al <sub>2</sub> O <sub>3</sub> ).	Ferric oxid (Fe <sub>2</sub> O <sub>3</sub> ).	Alumina (Al <sub>2</sub> O <sub>3</sub> ).	Ferric oxid (Fe <sub>2</sub> O <sub>3</sub> ).
J. A. Le Clerc.....	6.56	2.54	6.43	2.67
Andrew J. Patten.....	7.12	2.73	7.17	2.68
S. C. Dinsmore.....	<sup>a</sup> 7.45	<sup>a</sup> 3.35	<sup>a</sup> 7.45	<sup>a</sup> 3.35
O. M. Shedd.....	6.74	2.66	6.74	2.66
H. D. Haskins.....	6.64	2.63	6.69	2.58
Average.....	6.77	2.64	6.76	2.65

<sup>a</sup> Not included in average.

## COMMENTS BY ANALYSTS.

J. A. Le Clerc found that it was necessary to redigest the ash residue with hydrochloric acid in order to remove all of the iron present. In Method C some organic matter seemed to interfere with the end point of permanganate titration.

Andrew J. Patten used an approximately hundredth-normal solution of permanganate solution, this being preferred on account of the small amount of iron present.

O. M. Shedd found objection to the determination of iron by Method B in that it takes a long time to dissolve the oxids in the sulphuric acid solution, and at this point recommended the fusion of the oxids with potassium hydrogen sulphate.

The referee is of the opinion that a weaker solution of permanganate is preferable to the one recommended.

The average results obtained by the two methods agree very closely and indicate but little choice in method of procedure.

The variations obtained between the various chemists in the results reported may be due to the method of standardizing the permanganate solution. The referee has used for this purpose a solution made from iron of a known composition furnished by the Bureau of Standards, Washington, D. C. The personal equation must also enter into cooperative work of this nature to a greater or less extent.

## RECOMMENDATIONS.

In view of the fact that the two methods gave results agreeing within 0.01 of a per cent, the referee feels justified in recommending the one which involves the least manipulation. The following is therefore recommended as an official method for the separation of iron and aluminum in inorganic plant constituents:

Use an aliquot part of solution A corresponding to 0.2 to 0.5 gram of ash for the determination. After removing the phosphoric acid, place the filtrate from the precipitate of phosphomolybdate, consisting of the nitric acid solution of molybdic acid, ferric oxid, alumina, lime, and magnesia, in a beaker and cautiously neutralize with ammonia, care being taken that the temperature does not rise above 40° C., and that the alkali is added only in slight excess; allow to stand in a warm place until the precipitate completely settles. Filter the clear supernatant fluid, wash the precipitate a couple

of times with hot water by decantation before transferring it to the filter, wash four or five times on the filter with hot water. Dissolve the precipitate through filter with weak, hot nitric acid (1 to 5), reprecipitate with ammonia, filter and wash in the same careful manner. Dry, ignite, and weigh the precipitate as ferric and aluminic oxids.

Transfer an aliquot part of the original solution A, corresponding to 0.2 to 0.5 gram of ash, to an Erlenmeyer flask and evaporate with 10 cc of sulphuric acid on a hot water or steam bath until all of the hydrochloric acid is expelled; dilute with distilled water to original volume and reduce the iron to the ferrous state by adding iron-free metallic zinc (about 5 decigrams at each addition) until the solution is completely decolorized and the iron is all reduced. Cool and estimate the iron by standard solution of potassium permanganate. Deduct the per cent of ferric oxid obtained from the per cent of ferric and aluminic oxids to obtain the per cent of alumina. Use a fiftieth-normal solution of potassium permanganate standardized by a solution of metallic iron of known composition.

It is also recommended that further work be done with the peroxid method for the determination of total sulphur in plants and plant products, as suggested by the committee at the last association meeting. Lack of time has prevented the referee from taking up this important subject during the past year.

## REPORT ON MEDICINAL PLANTS AND DRUGS.

By L. F. KEBLER, *Referee*.

Much activity has been shown during the past year by both federal and state officials charged with the enforcement of the various laws governing medicinal agents. The drugs studied were both imported and domestic. Many interesting results have been observed, a few of which will be noted in the referee's report. The feature standing out most prominently is the lack of standards and recognized methods for detecting the presence and determining the amounts of many active medicinal agents, and from the nature of some of these agents no satisfactory methods will probably be provided in the near future. Even some of the methods available and the standards set are found wanting. In this connection it is desirable to call attention to certain features of the Pharmacopœia. A plant product is described, and in some cases a standard relative to alkaloidal content is prescribed, but in many instances no provisions are made for excluding or permitting the presence of any foreign materials, such as stems, sticks, etc. It is a very common experience to meet with importations of leaves containing large quantities of these impurities. The same holds true with other commodities, such as cubeb berries, in which frequently a large percentage of stems and twigs is found, together with unripe or overripe berries, and the question arises, To what extent is it permissible for these materials to be present? It is contended by producers and importers that a standard precluding the presence of these foreign agents would be purely theoretical, academic, if you please, and has no standing in the business world. On the other hand, it is well known that the medicinal value of a preparation is enhanced or depreciated in value in proportion to the quantity of these foreign agents present. For example, the per cent of alkaloid material present in belladonna root will be lowered in proportion, other things being equal, to the amount of adulterant present. In other words, it is depreciated at least in medicinal value proportionately to the foreign material present, and to what extent these foreign bodies unfavorably influence the medicinal action of a drug in which they are found is unknown.

Another feature is the amount of sand or incidental earthy matter present. For example, normal senna leaves do not contain to exceed 10 per cent of sand and other inorganic material, but it is not uncommon to meet with siftings, sweepings, etc., of senna containing from 20 to 35 per cent of such impurities. There is no provision in the Pharmacopœia setting an ash limit to a product of this character, but it is reasonable to expect that sand does not constitute a material part of a normal product used

for medicinal purposes. How many would be willing to administer to children compound licorice powder prepared with senna leaves containing 25 per cent of sand?

Probably no other drug has caused so much annoyance and dissatisfaction during the past year as asafœtida. When the drug sections of the federal law were put into effect at the various ports it was found that this commodity was brought in containing various amounts of alcohol-soluble material. No important quantity of exceedingly inferior material was offered, and for the time being no detentions were made, even though this drug was somewhat below the strength prescribed by the U.S.P. for alcohol-soluble material. It soon developed, however, that importers were bringing in successive lower grades of this product; for example, the alcohol-soluble material diminished gradually from 40 to 30 to 25 and to 20 per cent, and one consignment was offered containing, according to the declaration on the containers, as low as 15 per cent of this material, while one case of this consignment was found to possess only a trifle over 6 per cent of such alcohol-soluble material. The Pharmacopœia also prescribes an ash limit of 15 per cent. The virtue of asafœtida resides largely, if not exclusively, in the alcohol-soluble material, and it would therefore seem that the ash limit should be liberal. If an importation were offered containing on the average 50 per cent or more of asafœtida alcohol-soluble material, but the ash was materially above the limit prescribed by the Pharmacopœia, such an importation should not be considered illegal.

Attention is also directed to another drug, namely, copaiba. During the past year large quantities of this product were imported and correspondingly large examinations were made at the ports, particularly New York. It will be recalled that the test as originally prescribed by the committee of revision of the U.S.P. was modified at the earnest solicitation of many dealers in this commodity. The result is that the new method is so unreliable and unsatisfactory as to permit the entry of copaiba containing at least 25 per cent of Gurjun balsam, the common agent used for its adulteration. A question arising in connection with this commodity is, Shall the definition given relative to copaiba as contained in the U.S.P. be strictly adhered to, or shall we recognize under the name copaiba, qualified or otherwise, any other commodity which is derived from other sources than those definitely prescribed by the above authority? For example, there is constantly offered for importation a product known as African copaiba, which is derived from an entirely different geographical source than the commodity recognized by the Pharmacopœia. It is well known that the African oleoresin differs materially in composition and therapeutic action from the copaiba recognized by the U.S.P. African copaiba certainly is not copaiba within the meaning of the pharmacopœial definition for this commodity.

Another problem requiring attention is the dilution of certain drugs with inert substances. The Pharmacopœia prescribes a lower limit of alkaloidal content for certain potent drugs and it has developed that millers are adding to alkaloidal drugs assaying above the lower limit prescribed by the Pharmacopœia such inert material as powdered olive stones so as to reduce the alkaloidal content to the lowest limit prescribed by the above authority. There is nothing in the Pharmacopœia that would indicate that such a practice was contemplated or recognized. The committee undoubtedly was familiar with the fact that alkaloidal drugs frequently contain a larger amount of alkaloidal material than the lower limit prescribed, but only in one case, and that is opium, is there a specific provision made for the addition of a foreign inert material so as to reduce the product to a strength conforming to both a lower and an upper limit. This is an important question and requires adjustment in the near future.

Attention has also been directed to the fact that there are many commodities on the market which probably owe their virtues to their alcoholic content; for example, there are a number of so-called (for want of better names) medicinal wines, bitters, etc., which contain only a dash of some medicinal substance such as extract of cin-

chona, gentiana, etc., or very small amounts of one or more of the cinchona alkaloids. Mixtures of quinin and whisky are cases in hand. One of the products examined was found to contain not more than one-fortieth of a grain of total alkaloidal matter to an ounce of the product, and even then the alkaloidal matter consisted only in part of quinin. Without informing a prospective consumer or partaker, he, as a rule, would not detect any abnormal odor or taste, excepting the one imparted by the so-called whisky itself. One of the arguments frequently used to justify the existence of products of this character is that the National Formulary, a standard quoted by the food and drugs act, recognizes preparations of a similar type. The preparation referred to most frequently is beef, wine, and iron. The point raised is an exceedingly important one and requires adjustment. If it is permissible to add simply enough of an agent to merely suggest a certain physiological action, be it ever so remote, primarily for the purpose of using the name of a substance possessing recognized medicinal properties, in conjunction with the trade name of a commodity, one helpful feature of the law will be largely negatived and an increased number of so-called medicinal products of the most absurd character can be placed upon the market.

As before stated, there are many drugs for which we have no satisfactory chemical methods for determining whether or not given samples are active or inert. In some few cases it has been possible to employ animal experimentation. The drugs amenable to this form of study are digitalis, cannabis indica, strophanthus, etc. The methods at present available appear to give fairly satisfactory results in the hands of experienced operators, but there is much to be done before it can be definitely stated whether or not a given consignment possesses sufficient medicinal properties. One specific case in this connection should be noted, namely, digitalis leaves. This product is of such great importance to the medical practitioner for the treatment of certain heart affections that nothing relative to this drug and its preparation should be left to chance. No less an authority than Doctor Dixon, of England, says: "For my part I unhesitatingly express the belief that many hundreds of patients die annually from digitalis and allies not possessing the virtues which are required of them."

At the meeting of the association last year the referee urged the appointment of a number of associate referees for the purpose of studying certain features which need careful investigation; for example, it is absolutely necessary to have an intimate acquaintance with the macroscopical and microscopical features of crude plant products. Satisfactory methods are also wanting for analyzing the many mixtures containing modern synthetic chemicals, used for the treatment of headache and numerous other affections. We are in possession of fairly reliable methods for determining and estimating morphin in certain combinations or if present in considerable amounts, but it is quite another matter to detect and estimate this agent when present in very minute quantities in mixtures containing various solvents, and much study will be required before this single item is placed upon a footing which will be absolutely reliable. During the past year some work has been attempted along this line with cocain. It may at first appear to be a very simple matter to detect the presence and estimate the amount of cocain in mixtures, but when it is remembered that there are quite a number of products which respond to one or more of the tests laid down for detecting cocain the difficulties can readily be appreciated. Careful, thoroughgoing investigations on all these points are greatly needed, and until more work is done it will be difficult to satisfactorily enforce some features of the federal and state laws dealing with these products.

State officials are making frequent requests for information as to methods of analysis and standards for certain products. The referee has been actively engaged on both of these lines of work and has now in preparation standards for certain products for which no standards of a satisfactory character exist, and in cases where the existing standard is somewhat deficient it is the intention to add certain features which will

enable all workers throughout the United States engaged in the investigation of these products to arrive at just conclusions relative to their quality. For example, it is intended to fix an upper limit of the amount of foreign material that may be present in a leaf described by the United States Pharmacopœia and to provide an ash limit for certain drugs. Considerable progress has also been made relative to testing existing analytical methods and formulating new methods for examining certain commodities.

In order to facilitate the investigation, and in harmony with the instructions of the association, the referee appointed several associates to take up specific features of the work, whose results will be given in separate papers.

## A PRELIMINARY STUDY OF THE MICROCHEMICAL ANALYSIS AND IDENTIFICATION OF ALKALOIDS.

By B. J. HOWARD and C. H. STEPHENSON.

The large number of alkaloids used at present in drugs as well as the increasing number of synthetic products being placed on the market has made felt the want of additional means for their identification. Microchemical methods have been suggested and used to a limited extent by such workers as Wormley, Barth, and Behrens, but the principal application of the method has been rather for the purpose of localizing the alkaloid in the tissues, by such investigators as Errera, Maistriau, Clautriau, Bolling, and others.

At the suggestion of the Chief of the Division of Drugs this investigation was taken up by the Microchemical Laboratory and a study of several alkaloids begun. Only a preliminary report of progress can as yet be made, and the field has extended itself in many directions as the work progressed. The investigation as originally outlined anticipated several lines of work, among which the following might be mentioned:

(1) The normal reaction of each alkaloid with each of the various reagents which are known to be of service with one or more of them. This involves a study of dilution, the dilution of the alkaloid which will respond to the test and also the weight limit of alkaloid which will give positive tests with the reagent. It also involves a study of the forms of crystals produced at the various dilutions, the conditions for producing the reaction, or the manipulation, the determination of melting points, photographing the crystals as a matter of record, and in some cases the measurement of crystal angles.

(2) A study of the influence upon the reaction of another alkaloid present than the one sought.

(3) A study of the influence upon these reactions of such substances as glycerin, sugar, starch, oils, and fats, gums, waxes, and other compounds likely to be found in drugs, and from traces of which it is often difficult to remove, for testing, small traces of alkaloids in some medicinal preparations.

(4) The adaptation of alkaloidal purification methods for use microchemically so as to permit minute quantities of the alkaloids to be separated and prepared for testing.

(5) The developing of an analytical scheme for systematically identifying microchemically the various alkaloids present in unknown mixtures. This last can only be accomplished after a considerable number have been studied and compared.

During the last year the work has been practically confined to the first two lines, which naturally constitute the foundation of the whole investigation. The alkaloids studied comprise a list of about forty, besides two or three salts of two of them, most of which were obtained through the Division of Drugs. They were commercial specimens and apparently of average purity. The list also embraces several synthetic compounds as well as the more common natural alkaloids. Among the natural alkaloids or their salts studied might be mentioned the following: Cocain, codein, atropin, cinchonin, morphin, papaverin, narcein, caffen, strychnin, tropacocain, hydrastin, coniin, berberin, solanin, etc., while among the synthetic bodies studied are anæs-

thesin, beta-eucain, holocain, gujasanol, and acoin. The dilution of the alkaloid in the solvent in many cases has a most marked effect upon the form assumed by the precipitate. There is always a limit beyond which the dilution of the product is too great for crystallization to take place, while on the other hand the concentration may be so great as to cause too sudden precipitation and an unsatisfactory product results. In this work dilutions of 1:100 or 1:200 were most frequently tested. Other dilutions would possibly have given crystalline products where only noncrystalline products have thus far been obtained or where no reaction at all has been noted.

The reagents used embraced a list of more than ninety compounds or mixtures and included the standard reagents and as far as known, the special alkaloidal reagents with the exception of two or three which have recently been brought to the authors' attention. Thus far crystalline precipitates have been obtained in about 400 combinations. Noncrystalline deposits resulted in nearly 600 other combinations, but their usefulness in identification is very limited and they can usually only be employed as corroborative tests.

Unfortunately some of the well known alkaloidal reagents, though giving reactions with most of the alkaloids, produce only noncrystalline precipitates. As ordinary analytical tests they may be satisfactory, but as microchemical reagents they leave much to be desired. To the analytical chemist they serve a good purpose as indicating alkaloidal presence, but rarely its identity. This is shown in the following examples: Mayer's reagent, 11 crystalline, 23 noncrystalline; Kraut's reagent 10 and 33 and Marme's reagent 11 and 25, respectively.

Picalonic acid gave 21 crystalline precipitates out of 37 positive reactions, but the forms unfortunately are in most cases too much alike to be of much service for identification. The alkaloids studied showed a great diversity in the character of the precipitate formed, as is seen from the following examples, which serve to illustrate the extremes, the first four giving a high number of crystalline forms, the last five giving a high number of noncrystalline.

*Character of precipitate obtained with different alkaloids.*

Alkaloid.	Crystalline.	Noncrystalline.	Alkaloid.	Crystalline.	Noncrystalline.
Strychnin.....	36	3	Apomorphin.....	6	35
Berberin.....	25	.6	Papaverin.....	9	32
Tropacocain.....	22	2	Hydrastin.....	1	32
Anæsthesin.....	20	7	Solanin.....	1	29
Acoin powder.....	2	40			

With piperin, sanguinarin, emetin, and apocodein noncrystalline precipitates only have been obtained thus far, though it may be that by some change of manipulation crystals may yet be produced.

The melting point of the products is likely to be of service at times in establishing the identity of certain compounds though some of the precipitates apparently are too unstable for this test. For this purpose, however, an apparatus which had been devised in the Bureau of Chemistry for use on the stage of the microscope has been tested with promising results. It allows of the microscopic examination and determination of the melting point of an individual crystal in a mixture of various kinds either with plain or polarized light. In some crystals, especially some of the compact spherical forms, this last point is an important means of telling where melting begins, since as soon as a crystal melts it loses its polariscopic activity, and as all systems, except those belonging to the regular system, are active this feature can be used to advantage in determining the point where melting begins and where it ends even on small crystals.



The alkaloid thus far most thoroughly examined is cocain. Crystalline deposits have been obtained with each of the following eleven reagents, viz, palladous chlorid, platinum chlorid, gold chlorid, picric acid, chromic acid and hydrochloric acid, potassium dichromate and hydrochloric acid, potassium permanganate, potassium chromate, sodium carbonate, ferric chlorid, and potassium hydrate or sodium hydrate; noncrystalline deposits were obtained with chlorzinc iodid, picralonic acid, Mayer's reagent, phosphomolybdic acid, phosphotungstic acid, Kraut's reagent, Wagner's reagent, barium mercuric iodid, and potassium cyanid.

The following observations were noted concerning the various reactions with cocain in which crystals were produced:

*Palladous chlorid.*—This is one of the most characteristic tests for cocain, though not quite so sensitive as gold chlorid. The crystals vary in form greatly, according to the conditions of precipitation. There is at first formed, except in very dilute solutions (1:300 and up), an orange-colored amorphous-like or oily precipitate from which, on standing, crystalline forms of golden brown color are produced. One of the most common forms is that obtained with a 1:100 dilution, when feathery crystals are formed which have a strong tendency to twin. With a solution of 1:20 a dense precipitate is thrown down, out of which hexagonal plates are at first formed and frequently followed later by sheaf-like clusters of fine-pointed acicular crystals. A dilution greater than 1:500 gives crystals only with difficulty, crystallization being induced by rubbing the slide with the glass stirring rod. The limit of the test is 0.2  $\mu$ gr.

*Platinum chlorid.*—With a 1:20 solution a dense white precipitate is formed and quickly followed by the production of very narrow feathery crystals—many times twinned so as to resemble a bird with outspread wings. Clusters of more than two are also abundant. If the reagents are mixed slowly the crystals are more like those of greater dilution. With a 1:100 dilution the feather type is much more prominent, the secondary branches being well developed into frost-like forms. With 1:1,000 solutions either short thick crystals are formed or else plate crystals twinning in a most characteristic manner are produced. The dilution limit is about 1:4,000, and the limit in 1:1,000 is 0.2  $\mu$ gr.

*Gold chlorid.*—This is the most sensitive reagent for cocain so far found. At 1:100 feathery frost-like crystals are produced, together with some nearly smooth star-like aggregates. At 1:1,000 the form is much the same, but the branches usually bear a rough outline. Diamond plates are also produced. At 1:4,000 a cross-like form predominates, the cross-bar being short. A few rosette crystals frequently are present. Crystals can be obtained in dilution up to 1:20,000 and the limit of the test for dilutions of about 1:3,000 is 0.033  $\mu$ gr.

*Picric acid.*—This is a good reagent for dilutions up to 1:800, though the crystals produced are not very characteristic for this alkaloid. They are produced in spherical rosettes (or sheafs) of fine lemon-yellow acicular forms. The reaction takes place quickly, and no difficulty is experienced in producing them nearly to the limit of dilution. At 1:300 the limit is 0.2  $\mu$ gr.

*Potassium permanganate.*—With cocain, solutions up to a dilution of 1:700 give purple-colored square plates, or aggregates of this form. Vigorous rubbing of the slide is often necessary to start the crystallization, which then proceeds readily. When they begin to crystallize spontaneously, the plates are sometimes deposited in spherical aggregates. The limit at 1:400 is 2  $\mu$ gr.

*Chromic acid and hydrochloric acid.*—This test is made by adding a small drop of 5 per cent chromic acid solution to the test drop. A precipitate is formed which on stirring disappears (if too much has not been added). A small drop of strong hydrochloric acid is added and a yellowish deposit is produced, which after rubbing of the slide should in a few moments be transformed into loose spherical clusters of an acicular crystal. This test appears to be one of the most uncertain because of the difficulty with which the crystallization is sometimes induced to begin in dilutions greater than 1:60. A concentration of 1:1,000 has produced positive results on standing several minutes. The limit appears to be for 1:100 about 3  $\mu$ gr.

*Potassium bichromate and hydrochloric acid.*—This test gives the same form of crystals as the chromic acid and the test is conducted in a similar manner. The limit of dilution is about 1:1,000 while at 1:100 the limit is 3  $\mu$ gr.

*Ferric chlorid.*—The crystals are spherical aggregates of rather coarse blade-like crystals with chisel-shaped ends. The limit of dilution is about 1:1,000 and in a dilution of 1:100 the limit is 3  $\mu$ gr.

*Potassium hydrate, or sodium hydrate.*—This produces a white amorphous precipitate which changes into crystals on standing or by rubbing the slide with a glass rod.

The crystals are rod-like, frequently with more or less chisel-like ends and a V-shaped recess extending backward into the crystal. There is a strong tendency to form coarse clusters up to about 15 branches. In open drops tree-like forms are frequent. For each of these reagents dilutions up to 1:1,000 give the reaction and the limit at 1:100 is 3  $\mu$ gr.

*Sodium carbonate*.—This gives a precipitate with cocain like that produced by potassium hydrate, both in the amorphous and crystalline forms. Limit of dilution is 1:1,000. In 1:100 solution the limit is 3  $\mu$ gr.

In order to determine the usefulness of some of the above tests when other alkaloids are present the palladous chlorid test was made on test drops to which had been added solutions of one of the following alkaloids, codein sulphate, atropin sulphate, heroin, dionin, acoin powder, cinchonin sulphate, hydroxylamin hydrochlorid, apomorphin hydrochlorate, narcotin, papaverin, brucin, narcein, morphin, thebain, gujasanol, orthoform (new), cinchonidia sulphate, quinidia sulphate, beta-eucain, holocain, caffein, quinin sulphate, strychnin, and tropacocain. In each case the crystals of the cocain compound were obtained and in the case of brucin, gujasanol, caffein, strychnin, and tropacocain, with which the palladous chlorid regularly gives a crystalline precipitate, it was found that when cocain was also present the cocain product was given in addition to that for the other alkaloid, though occasionally with modified form.

The foregoing serves to give an idea of the scope of the work undertaken, which it is hoped will be carried much further during the coming year.

## COOPERATIVE WORK ON HEADACHE MIXTURES.

By W. O. EMERY.

After making investigations of various suggested methods for determining the different constituents present in the many headache mixtures containing acetanilid and similar agents, a method was finally devised which proved quite satisfactory to the members of the Division of Drugs, and it was therefore decided to place this method in the hands of as many chemists interested in this line of work as could assist. A circular letter requesting cooperation was sent out, and a gratifying number responded, signifying their willingness to assist, eleven of whom sent in results. All who expressed a desire to cooperate were supplied with a sample of a mixture containing known amounts of acetanilid, sodium bicarbonate, and caffein, with the following instructions, the U. S. Pharmacopœia, eighth revision, as amended and corrected May 1 and June 1, 1907, being used as a basis for all calculations and reagents unless otherwise specified:

### SEPARATION OF CAFFEIN, ACETANILID, AND SODIUM BICARBONATE.

#### *Caffein.*

Weigh out about 0.3 gram of headache powder on a small (5.5 cm) tared filter,<sup>a</sup> wash with successive small portions of chloroform to the amount of about 30 cc, collecting the solvent in a 100 cc Erlenmeyer. Distil off chloroform by means of a small flame until only a few cubic centimeters remain. Add 10 cc of dilute sulphuric acid, then continue the distillation till all the chloroform has gone over, disconnect from condenser, heat gently, first on wire gauze to complete solution,<sup>b</sup> finally on a steam or hot-

<sup>a</sup> In cases of powder mixtures or tablets containing ground celery seed, much coloring matter, cinchona alkaloids, laxative or extractive principles other than acetanilid or phenacetin, it is our practice to shake out the latter by means of chloroform from dilute sulphuric acid solution.

<sup>b</sup> In case the preparation contains ground celery seed or certain oily principles, it sometimes happens that the acid solution does not become entirely clear at this point.

water bath until the contents of the flask have evaporated to about 3 to 4 cc. Cool, transfer by washing with water to a separatory funnel, so that the final volume does not greatly exceed 20 cc. Add four times the volume, or about 80 cc of chloroform, shake for some time vigorously, allow to stand until the chloroform clears perfectly, pass through a small dry filter into a dry 100 cc Erlenmeyer, distil off the solvent and use distillate for a second extraction, observing the same method of shaking, clearing, and filtering as above noted. Distil off chloroform to a small volume, transfer residue to a small tared beaker, or crystallizing dish, by means of a few cubic centimeters of chloroform. Allow to evaporate spontaneously, or if desired on a steam or hot-water bath to dryness, in the latter case partially covering the dish toward the end of operation with a watch glass in order to avoid possible loss from "popping." Cool in desiccator and weigh as caffeine, dry alkaloid.<sup>a</sup>

#### *Acetanilid.*

*First method.*—The acid solution remaining in the separator and containing anilin sulphate is run into a 100 cc Erlenmeyer, the filter through which the chloroform passed is washed once with a little water, allowing the latter to run into the separator. Rinse the latter thoroughly, adding the aqueous rinsings to the acid solution. Now, run in slowly and with constant agitation a standard solution of potassium bromid-bromate <sup>b</sup> to a faint but distinct yellow coloration. The number of cubic centimeters employed, multiplied by the value of 1 cc in terms of acetanilid, will give the amount of acetanilid present.

*Second method.*—The acid solution aforesaid is treated with successive small portions of sodium bicarbonate until an excess of this reagent is observed in the bottom of the separator. Add 50 cc of chloroform and 15 to 20 drops of acetic anhydrid, shake for some time vigorously, allow the chloroform to clear, then pass through the same filter used for the caffeine into a 100 cc Erlenmeyer, and distil off most of the chloroform. Use this distillate for a second shake out, clear, filter, and distil down to a small volume, transferring the residue and the subsequent chloroform washings to a tared beaker or dish precisely as in the case of caffeine. Allow the solvent to evaporate spontaneously or by means of a blast or fan, avoiding, however, undue heat.<sup>c</sup> Dry in desiccator over quicklime to constant weight.

Verify the final weight by means of titration with standard potassium bromid-bromate solution as in the first method. Heat the residue with 10 cc dilute sulphuric acid a half hour on the steam or vapor bath, cool, add 5 cc of water and titrate as directed above.

#### *Sodium bicarbonate.*

The residue left after the first treatment with chloroform is weighed when dry and represents very nearly the amount of sodium bicarbonate present. It may be more accurately estimated by titrating with tenth-normal sulphuric acid, using congo red as indicator, or it may be ignited with dilute sulphuric acid and weighed as sodium sulphate.

Calculate results in parts per 100.

<sup>a</sup> Should the caffeine not be colorless or nearly so, the residue is dissolved in about 10 cc of water, filtered, if necessary (in case oily matters are present), through a wet filter, the filtrate acidified with dilute hydrochloric acid, the caffeine precipitated with 15 to 20 cc of Wagner's reagent, allowed to stand a half hour, filtered, and the precipitate washed with a few cubic centimeters of same reagent, the filter, together with precipitate, transferred to separator, decolorized by means of sodium sulphite, and the caffeine finally extracted with chloroform.

<sup>b</sup> For this purpose the solution is prepared by adding bromin in slight excess to a concentrated aqueous solution of 50 grams caustic potash, the liquid diluted till the separated salts redissolve, boiled, to expel any excess of bromin, and finally made up to 1 liter. This solution is standardized with weighed amounts of acetanilid, or it may be so adjusted by further dilution that 1 cc is exactly equivalent to 1 centigram of acetanilid. For purposes of titration 1 to 2 decigrams are heated a half hour on the steam or water bath with 10 cc of dilute sulphuric acid.

<sup>c</sup> Acetanilid suffers appreciable loss when heated above 40°.

The results reported are tabulated as follows:

*Results obtained in the cooperative work on an acetanilid mixture.*

Analyst.	Caffein.	Acetanilid.		Soda bicarbonate.		Total. <sup>b</sup>
		Volumetric.	Gravimetric.	Volumetric.	Gravimetric.	
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
L. A. Brown, North Dakota.....	12.16	65.93		23.20		101.29
	10.93	66.10			23.50	100.53
	11.05	65.93			23.20	100.18
	10.40	66.10				
L. D. Havenhill, Kansas.....	11.50	63.00			25.10	99.60
	10.73	61.50			25.00	97.23
	11.33	63.40			24.90	99.63
	11.53	63.44			25.00	99.97
H. L. Schulz, Michigan.....	11.00	63.00			24.60	98.60
	11.00	62.00			24.80	97.80
H. A. Seil, New York.....	10.55	65.78			25.03	101.36
	10.49		65.26		25.11	100.86
T. F. Darling, New York.....	10.48	65.80			25.13	101.41
	10.62		67.72		25.13	103.47
E. L. Redfern, Nebraska.....	9.80	65.10			25.06	99.96
	10.06	64.58			24.93	99.57
	9.93	63.03	63.60		25.00	98.25
	10.20	63.21	63.80		24.93	98.64
E. M. Bailey, <sup>a</sup> Connecticut.....	10.90	64.53			25.07	100.50
	11.17	64.00			24.54	99.71
	11.30			25.93	25.57	
C. B. Morrison, <sup>a</sup> Connecticut.....	11.37			25.67	25.33	
	10.67	64.80		25.93	25.43	101.15
A. R. Mehrtens, California.....	10.30	64.53			24.99	99.82
G. E. Colby, California.....	10.00	65.33			24.79	100.12
W. O. Emery, Washington, D. C.....	10.23	64.79			24.92	99.94
	10.29	64.85			24.95	100.09
	10.01		64.68		25.01	99.70
Average.....	10.71	64.38	65.01	25.18	24.89	99.98
Maximum.....	12.16	66.10	67.72	25.93	25.57	103.47
Minimum.....	9.80	61.50	63.60	23.20	23.20	97.23
Difference.....	2.36	4.60	4.12	2.73	2.37	6.24
Known composition of acetanilid mixture (acetanilid, 453 parts; caffein (anhyd.), 70 parts; soda bicarbonate, 174 parts)....	10.04	64.99		24.96		99.99

<sup>a</sup> Reported by J. P. Street.

<sup>b</sup> In cases where two percentages for volumetric and gravimetric determinations of the same substance were reported, the mean of such percentages has been taken in computing the total percentage.

Owing to an ambiguity in the expression "dilute sulphuric acid" employed in the method under caffein, as also in the footnote *a*, page 100, for standard bromid-bromate solution, some of the workers quite naturally used the pharmacopœial strength, with the result that the acetanilid was not completely hydrolyzed. This undoubtedly explains the somewhat high results for caffein and the correspondingly low ones for acetanilid. The strength of acid intended and the one actually employed for this purpose in the Bureau of Chemistry is that ordinarily used in laboratory work and is made by diluting 1 part of concentrated sulphuric acid (whose specific gravity is not less than 1.826 at 25°) with 5 parts of water. From two to three hours' heating on the steam bath is usually required to completely hydrolyze the acetanilid.

Notwithstanding this ambiguity the results obtained are very gratifying, in view of the fact that the method is new and the workers have entered into a comparatively new field. The percentages of variation are so small as to almost warrant the referee in recommending it as a provisional method to the association. He believes, however, that the method should receive additional study, and so recommends. It is also recommended that additional mixtures be tested with this and such other methods as may be found desirable.

President Snyder introduced the Secretary of Agriculture with a few words of appreciation concerning the long-sustained attitude of the Secretary in fostering agricultural chemistry, especially the work of the official chemists, by making possible the close affiliation between the Department of Agriculture and the association. The Secretary then briefly addressed the convention, after which the reading of the drug reports was resumed.

#### THE NECESSITY FOR ANIMAL EXPERIMENTATION IN DETERMINING THE PURITY AND STRENGTH OF MEDICINAL PREPARATIONS.

By WILLIAM SALANT.

Experiments on animals have long been recognized in medical jurisprudence as a valuable adjunct to chemical and microscopical methods in the detection of poisons in animal tissues and fluid. Notwithstanding the improvements in the methods of analytical chemistry witnessed within recent years, tests on animals, or, as Kobert<sup>a</sup> terms it, "biological testing," is still resorted to in order to corroborate the findings of the analytical chemist in cases of suspected poisoning with alkaloids and other poisonous substances of plant or animal origin. The French chemist, Boutmy,<sup>b</sup> who made extensive studies on poisoning with alkaloids, concluded that in all cases in which the presence of an alkaloid in the body is suspected experiments on animals should be made for the purpose of confirming the results of chemical analysis.

The work of some investigators indicates that the biological method is in certain cases much more delicate than the chemical. Ranke<sup>c</sup> reports experiments on dogs which were given 0.1 of a grain of strychnin by mouth. Chemical examination of the organs of these animals failed to show the presence of strychnin, but when extracts of the same organs were injected into frogs tetanus followed. Falck<sup>d</sup> has shown long ago that one-twentieth of a milligram of strychnin was sufficient to induce tetanus in a medium-sized frog. It might be added that if smaller frogs are used the same effect may be obtained with one-eightieth of a milligram. Atropin is another example of a drug of which small quantities are sufficient to produce a physiological reaction. Only one-twentieth of a milligram is necessary to produce dilation of the pupil.

Likewise cocain, which produces characteristic effects on the mucous membranes, and by its action on the frog's pupil, can be identified, even when very small quantities are present in biological solutions.

Aconitin can be identified in milligram doses by its action on the tongue, eye, heart, and central nervous system. No chemical methods have as yet been devised by which such small quantities of this alkaloid can be detected. A striking illustration of the delicacy of the biological method is afforded by the work of Hunt.<sup>e</sup> In his investigations on the functions of the thyroid he has shown that mice fed for a few days with the extract of this gland acquire greater resistance to poisoning with acetonitrile. One milligram of the official dried thyroid fed to white mice daily for a few days may enable the animals to recover from double the dose of acetonitrile fatal to the controls. Seidell,<sup>f</sup> working under the direction of Hunt, found that forty to fifty times as much thyroid would be required to give the iodine test.

<sup>a</sup> Ber. deutsch. pharm. Ges., 1903, 13: 325.

<sup>b</sup> Ann. hyg. publique med. legale, 1880, [3] 4: 193.

<sup>c</sup> Virchow's Archiv, 1879, 75: 20.

<sup>d</sup> Vierteljahrschr. gericht. Med., N. F., 1874, 20: 198.

<sup>e</sup> J. Amer. Med. Assoc., 1907, 49: 240.

<sup>f</sup> Ibid.

Even more delicate are the methods employed in the study of immunity. As shown by the work of Meyer <sup>a</sup> and Uhlenhuth, <sup>b</sup> by means of the precipitin test the presence and nature of proteins may be ascertained in a dilution of 1:100,000. As is well known, the chemical tests for albumin are of no value in dilutions over 1:1,000, and it is not specific.

For the identification of some poisons and the standardization of certain drugs of vast therapeutic application experiments on animals are practically the only reliable method.

There are no satisfactory chemical methods for the identification of the saponins, but owing to their powerful hemolytic action and their effect on the heart and voluntary muscles their identification has become possible. According to Köbert, <sup>c</sup> picrotoxin can be identified by experiments on animals only. The detection of curarin by chemical tests is very unsatisfactory; by its action on the motor end organs, however, its identity can be established even when mere traces are present. Thus motor paralysis in frogs has been induced by injecting 0.005 milligram of curarin.

Adrenalin has been the subject of numerous investigations. On account of its powerful action and its extensive therapeutic application, the strength of the various preparations should be accurately known. A number of color tests have been proposed for this purpose, some of which are of doubtful value and some may be employed if properly controlled by tests on animals, which are very delicate. Meltzer and Auer <sup>d</sup> have shown that a drop of a solution of 1:120,000 dropped into the conjunctival sac of a rabbit causes blanching of the conjunctiva and dilation of the pupil. According to Ehrmann, <sup>e</sup> a reaction of the pupil of the excised eye of the frog may be obtained with 0.000025 milligram of this drug. Similar results were obtained by other investigators who worked on the pharmacology of the drug. Cameron's <sup>f</sup> experiments with this drug on rabbits have shown that 0.0003 milligram per kilogram will cause a rise of blood pressure.

Preparations of digitalis have been found to vary enormously in physiological activity. Frankel <sup>g</sup> states that the strength of the tincture varies from 200 to 400 per cent, and the infusion varies from 100 to 125 per cent. In a recent article Lutzkaja <sup>h</sup> states that the infusion of digitalis he examined was only 60 per cent of the strength represented by the firm which prepared it. Barger and Shaw, <sup>i</sup> in England, examined nine tinctures of this drug, and found a variation of 75 per cent in their strength. These authors made a comparative study of Keller's method and the physiological test on an artificial infusion of digitalis made by adding a known quantity of the drug to a mixture of hay and chaff. This was extracted with 60 per cent alcohol. Chemical analysis of the extract showed the presence of 0.1 per cent of digitalis, whereas the mixture contained 0.4 per cent. The same extract was tested on frogs, however, and found to contain approximately 0.4 per cent.

In the case of some drugs the physiological test above must be relied upon, no chemical method having as yet been devised for their identification or quantitative determination. Cannabis belongs to this category. Houghton and Hamilton, <sup>j</sup> in a recent article, state that previous to the adoption of this test, preparations of the

<sup>a</sup> Lancet, 1900 (2), p. 98.

<sup>b</sup> Deutsch. med. Wochenschr., 1900, 26: 734.

<sup>c</sup> Loc. cit.

<sup>d</sup> Centrbl. Physiol., 1904, 18: 317.

<sup>e</sup> Arch. exp. Path. Pharmak., 1905, 53: 97.

<sup>f</sup> Proc. Roy. Soc. Edinburgh, 1905, 26: 161.

<sup>g</sup> Ther. Gegenwart, N. S., 1902, 4: 112.

<sup>h</sup> Arch. intern. pharmacodynamie, 1908, 18: 77.

<sup>i</sup> Yearbook of Pharmacy, 1904, p. 541.

<sup>j</sup> Ther. Gazette, 1908, 32: 26.

drug were so unreliable that hospital physicians used to experiment on patients with samples of this drug before placing an order for it. Since experiments on animals with this drug have been introduced, the practice of testing the preparations on human beings was abandoned.

Ergot is another drug whose activity can at present be determined only by experiments on animals. Crawford,<sup>a</sup> who made a study of chemical tests, came to the conclusion that they are of no value, while its action on the cock's comb after subcutaneous injection, or on the isolated uterus of the cat, is characteristic and may therefore be used to advantage in its identification or in determination of the strength of the preparation.

## REPORT ON INSECTICIDES.<sup>b</sup>

By C. C. McDONNELL, *Referee*.

A study of methods for the examination of insecticides and fungicides was taken up by the association only ten years ago at the fifteenth annual convention, when the first referee on this subject was appointed.

For the two years following this no analytical work was reported, but methods were compiled and suggested for the examination of this class of materials which were then most important, and these were adopted provisionally. All of them have been tested since that time and those proving of value have been officially adopted by the association. A number however have since been replaced by more rapid and accurate methods. As the list of substances used as insecticides and fungicides increases, as it is constantly doing, new methods must be devised and changes in old methods introduced in order that they may be adapted to a particular material.

At the present time the most important of these is lead arsenate, and it is to methods for the examination of this substance that considerable study is now being given. The work as carried out this year has been largely along the line of the recommendations of the referee last year, which were adopted by the association. In addition a modification proposed by the present referee for the determination of total arsenic oxid in London purple has been given a trial.

### WORK AS OUTLINED.

(1) A comparison of the provisional methods for London purple given in Bureau of Chemistry Circular 10, revised, the method as modified by Davidson, given in the Proceedings of the twenty-second annual convention of the association, also in Bureau of Chemistry Bulletin 107, revised, and the modified method as proposed by the present referee.

(2) A further study of the precipitation method for soda-lye, using fifth-normal acid instead of half-normal, also with and without removal of the barium carbonate precipitate before titration.

(3) Determination of formaldehyde in strong solution by the provisional hydrogen peroxid method, and on dilute solutions by the cyanid method to determine the amount of dilution necessary.

(4) Further test of the Avery method for the determination of sulphur in sulphur dips.

(5) A continuation of the study of the methods for lead arsenate proposed by Haywood (Proceedings 1906, Bulletin 105, p. 165) and tried last year.

Samples were sent to five chemists, who had expressed a willingness to cooperate in the work, and more or less complete reports have been received from three of these.

<sup>a</sup> Amer. J. Pharm., 1908, 80: 326.

<sup>b</sup> Owing to the illness of the referee, this report was not ready for presentation to the convention at the time of its meeting. Through the courtesy of the association the referee was permitted to present the report at a later date for insertion in the Proceedings.

## LONDON PURPLE.

Owing to the considerable variation in results and the difficulties encountered in the carrying out of the present methods for the determination of total arsenic oxid in London purple, methods for this substance have been receiving considerable attention from the association for several years. The three principal objections to the methods thus far proposed are: (1) The difficulty in reading the end point when using up the excess of liberated iodine with sodium thiosulphate after the reduction of the arsenic oxid to arsenious oxid. (2) The difficulty in reading the end point in the final reaction on adding the standard iodine solution. (3) The great tendency to foaming on adding sodium carbonate to neutralize the acid. Both the first and second are caused entirely, and the third largely, by the great amount of organic matter (dye) contained in London purple. Several methods have been proposed, which are more or less successful, for overcoming these difficulties. The two which are most used, however, have not given satisfactory results in the hands of all the analysts using them, in many cases the results running several per cent too low. The modification proposed by the referee and tried this year overcomes these difficulties and renders the determination of total arsenic oxid much easier, particularly for one who has not had considerable experience with the present methods.

In the following tables are given the results obtained by the different analysts, followed by their comments:

## TOTAL ARSENIUS OXID.

Method I is a provisional method and may be found in Bureau of Chemistry Bulletin 107, revised, page 28. Method II may be found on page 29 of the same bulletin. The results are very satisfactory, Method II appearing to give slightly lower figures.

*Total arsenious oxid (As<sub>2</sub>O<sub>3</sub>).*

Analyst.	Method I.	Method II.
	<i>Per cent.</i>	<i>Per cent.</i>
R. J. Davidson, Blacksburg, Va. ....	22.10	21.90
R. W. Thatcher, Pullman, Wash. ....	21.91	21.90
	22.45	21.99
	22.37	21.69
C. D. Woods, Orono, Me. ....		22.01
		22.01
		22.38
		22.38
		21.74
		21.74
C. C. McDonnell, Bureau of Chemistry. ....	22.07	21.90
	22.23	21.83
	22.26	21.86
Average. ....	22.20	21.93

## TOTAL ARSENIC OXID.

Method I is a provisional method and may be found in Circular 10, revised, and also in Bulletin 107, revised, of the Bureau of Chemistry, page 28. Method II may be found in Circular 10, revised. Method III is Davidson's modification of Haywood's method for removing a part of the coloring matter and may be found in Bulletin 107, revised, where it is designated as Method II, Provisional (p. 29).

Method IV is that proposed by the referee and is as follows:

Place 2 grams of the sample in a 200 cc graduated flask, add 5 cc of concentrated nitric acid and 20 cc of concentrated sulphuric acid. Place on a hot plate or over low flame and heat nearly to boiling; after ten or fifteen minutes add powdered sodium nitrate, in small quantities at a time, until all organic matter is destroyed and the solution is colorless. Cool, add about 50 cc of water (to decompose any nitro-sul-



phuric acid formed), and heat to boiling till nitric acid fumes are all expelled. Cool, make up to mark with distilled water, mix thoroughly, filter through a dry filter and take 50 cc of the filtrate (0.5 gram) for the determination of arsenic oxid. Transfer this 50 cc portion to a 400 cc Erlenmeyer flask, dilute to about 100 cc with water, add 2 to 3 grams of potassium iodid and 5 cc of concentrated sulphuric acid, heat to boiling and evaporate to about 40 cc. Cool, dilute to 150 cc to 200 cc and add approximately tenth-normal sodium thiosulphate just to disappearance of color caused by the free iodine. In case the solution is slightly colored from iron or incomplete oxidation of the organic matter, add the thiosulphate until nearly colorless, then add a few drops of starch paste and continue adding the thiosulphate slowly until the blue color just disappears. The exact end point can easily be obtained in this way. Neutralize immediately with sodium carbonate, make slightly acid with dilute sulphuric acid, and when all lumps of sodium carbonate are dissolved add sodium bicarbonate in considerable excess. Titrate with twentieth-normal iodine solution in the usual way, using starch solution as indicator. Subtracting from this the number of cubic centimeters of iodine solution corresponding to arsenious oxid as determined by Method I, gives the number of cubic centimeters of iodine solution corresponding to the arsenic oxid ( $As_2O_5$ ) in 0.5 gram of the sample.

*Total arsenic oxid ( $As_2O_5$ ).*

Analyst.	Method I.	Method II.	Method III.	Method IV.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
R. J. Davidson, Blacksburg, Va.....	18.69	18.76	18.55	18.63
R. W. Thatcher, Pullman, Wash.....	17.53	17.50	15.64	18.29
			15.56	18.06
			15.83	.....
			15.55	.....
			15.95	.....
C. D. Woods, Orono, Me.....			15.15	.....
			15.50	.....
			15.75	.....
			13.79	.....
			15.15	.....
C. C. McDonnell, Bureau of Chemistry.....	19.34	18.29	17.74	19.29
	19.73	18.23	18.04	19.42
	19.52	18.42	17.90	19.30

COMMENTS OF ANALYSTS AND DISCUSSION.

*R. J. Davidson:* There is no great difficulty in working the London purple by Method IV for arsenic oxid. It is perhaps a little more troublesome and you have to use another method for getting the arsenious oxid. I believe Method III, provided it gives satisfactory results, is the simplest method, both determinations being made from the same weighed sample.

*R. W. Thatcher:* Davidson's modification (Method III) makes the end point somewhat easier to determine. The modification suggested by the referee does not seem to me to offer any advantage over the official method, since the yellow color left after oxidation with nitrate mixture obscures the end reaction as badly as does the original color and has the disadvantage of requiring a separately weighed sample for the determination of the arsenic in arsenious form.

*C. D. Woods:* I would emphasize the fact and recommend that it be included in directions for insecticide work, that this kind of work is difficult for a beginner and that several preliminary determinations should be run before a man new to this work attempts to report results.

As has been the case in previous years, the results on arsenic oxid are very unsatisfactory, there being a difference of over 4 per cent between the highest and lowest determinations by Methods I and III, and over 2 per cent difference by the same method by different analysts. Methods II and III give lower results than Method I, but there does not appear to be any uniformity in the amount that these methods fall short, the determinations made by different analysts and even those by the same analyst at different times sometimes agreeing with those made by Method I and at others showing a variation of several per cent. Why this is so has not as yet been determined, but is under investigation. It is the referee's opinion that on precipi-

tating the coloring matter with sodium carbonate a varying amount of arsenic is carried down in the precipitate.

The determinations made by Method IV, while not agreeing as closely as might be desired, are close enough to justify a more extended trial of the method in the hands of different analysts. The writer has found it very satisfactory and, when properly carried out, a perfectly clear solution can almost always be obtained. Of course it is desirable to be able to determine both forms of arsenic on the same solution, but if it is found that this can not be done accurately this objection to the method becomes of minor importance.

#### LEAD ARSENATE.

The methods used for lead arsenate were proposed by Haywood at the meeting of the association in 1906 and were tried last year. They may be found in Bureau of Chemistry Bulletin 105, page 165; also Bulletin 107, revised, page 239.

The sample sent out for the work was made by the referee from C. P. di-sodium arsenate and lead acetate.

#### *Lead arsenate.*

Analyst.	Moisture.	Total arsenic oxid (As <sub>2</sub> O <sub>3</sub> ).	Total lead oxid (PbO).	
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	
R. J. Davidson, Blacksburg, Va.....	0.11	30.07	-----	
	.12	30.07	-----	
		30.14	-----	
R. W. Thatcher, Pullman, Wash.....	.09	29.97	-----	
	.09	30.39	68.58	-----
		30.24	68.48	-----
		29.63	<sup>a</sup> 66.31	65.96
		29.81	65.76	65.06
C. D. Woods, Orono, Me.....	.14	29.95	65.70	66.66
	.17	30.22	66.22	67.54
	.14	30.22	66.14	66.89
		30.22	65.94	67.17
C. C. McDonnell, Bureau of Chemistry.....	.15	29.47	65.85	66.95
	.17	29.80	66.58	67.32
		29.83	67.55	67.38
		29.60	67.35	67.40
			67.57	67.15
		67.58	67.10	

<sup>a</sup> Porcelain gooch used in all determinations of lead oxid.

#### DISCUSSION.

C. D. Woods states that in the determination of total arsenic oxid it was not found necessary to add thiosulphate to use up free iodine because if care is used in boiling the solution a colorless point is easily obtained.

The results on lead arsenate are not so uniform as might be desired, particularly on total lead oxid. However, the difference between the highest and lowest determination of arsenic oxid is only 3 per cent of the total amount present and for lead oxid 5 per cent of the total amount present. The method is certainly the best that has thus far been proposed and if carefully followed good results should be obtained.

#### SODA LYE.

METHOD I.—This is the precipitation method, and may be found in Circular 10, revised, page 8, and Bulletin 107, revised, page 31.

METHOD II.—This is the same as Method I except that the titration for hydroxide is made without removing the barium carbonate precipitate.

The acid potassium sulphate method was not submitted for trial, as satisfactory results had not been obtained by it in previous years and the association voted that it be dropped, as recommended by the referee in 1907.

As it was desired to send out samples containing considerable carbonate, and such were not at hand they were prepared as follows: The sample bottle was weighed and into this was weighed 2 grams dry sodium carbonate C. P. then, as rapidly as possible, 18 grams of commercial sodium hydrate. The bottles were then stoppered and sealed.

The analyst was directed to dissolve the entire content of the bottle in carbon dioxid-free water, make up to 2,000 cc and use 50 cc portions for the titrations (0.5 gram sample). The results submitted have been multiplied by two and reported in per cent in the following table:

*Soda lye.*

Analyst.	Method I.		Method II.	
	Sodium hydroxid (NaOH).	Sodium carbonate (Na <sub>2</sub> CO <sub>3</sub> ).	Sodium hydroxid (NaOH).	Sodium carbonate (Na <sub>2</sub> CO <sub>3</sub> ).
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
R. J. Davidson, Blacksburg, Va.....	84.40	13.25	84.80	12.72
R. W. Thatcher, Pullman, Wash.....	83.36	14.92	83.68	14.50
C. C. McDonnell, Bureau of Chemistry.....	83.76	14.40	83.52	14.74
	84.72	11.80	84.92	11.54
	84.72	11.80	84.92	11.54
Average.....	84.19	13.23	84.37	13.07

The results on sodium hydroxid are very good. As expected, Method II gives slightly higher results for hydroxid and lower on carbonate than Method I. The difference, however, is small. The referee determined carbon dioxid in a portion of the sample gravimetrically and found 11.62 per cent and 11.71 per cent calculated as sodium carbonate.

Using these two indicators in the same determination, as is done in this method, the tendency would always be to high results on sodium carbonate. Phenolphthalein, being more sensitive to acids, becomes colorless immediately when the solution is neutral, while with methyl-orange the acid must be in slight excess to develop the pink color, the excess required depending on the amount of indicator used and the depth of color titrated to. A blank should be made, using the same amount of water and indicator, and deducted in each case when methyl-orange is used. For the determinations in the second report in the table the analyst used normal acid. This may account for the results in sodium carbonate being high, as 0.1 cc normal acid is equivalent to over 1 per cent sodium carbonate, when operating on 0.5 gram of substance.

## FORMALDEHYDE.

Two samples were sent out for analysis, No. 1, a strong solution to be worked by the modified hydrogen peroxid method, and No. 2, a dilute solution to be worked by the cyanid method, both found in Bulletin 107, revised, page 33.

*Formaldehyde.*

Analyst.	Sample No. 1.	Sample No. 2.
	Method I.	Method II.
	<i>Per cent.</i>	<i>Per cent.</i>
R. J. Davidson, Blacksburg, Va.....	{ 36.81	{ 3.92
	{ 36.71	{ 3.98
R. W. Thatcher, Pullman, Wash.....	{ 36.64	{ 3.92
	{ 36.46	{ 3.83
C. C. McDonnell, Bureau of Chemistry.....	{ 37.00	{ 3.70
	{ 36.93	{ 3.98
	{ 37.00	{ 3.84
	{ 36.93	{ 4.02
Average.....	36.76	3.90

## COMMENTS AND DISCUSSION.

R. J. Davidson says: "I believe it would be well to state the amount of dilution necessary in Method II and not say, as the method does, 'a weighed quantity of the dilute formaldehyde solution.' The directions should be more specific."

The results on formaldehyde are very good. Method I is an excellent method for strong solutions, and Method II for dilute solutions, containing preferably not over 5 per cent. Even solutions of the latter strength must be diluted before making the determinations.

The referee is in favor of the recommendation made last year and referred to again in Mr. Davidson's report, that more specific directions should be given this method. If, instead of the words "a weighed quantity of the dilute formaldehyde solution," line 8, the following were inserted, "a weighed quantity of the formaldehyde solution containing not over 2 cc of a 1 per cent solution or the equivalent," it would make the method clearer and sufficiently explicit.

## SULPHUR DIPS.

The method is that of Avery and is given in Circular 10, revised, also Bulletin 107, revised, page 34. The sample submitted for analysis was prepared in the laboratory by boiling together lime and sulphur according to the regular formula for the lime-sulphur spray mixture.

*Sulphur dips.*

Analyst.	Date of analysis.	Weight of sulphur.
	1908.	<i>Gram per cc.</i>
R. J. Davidson, Blacksburg, Va.....	August 18.....	0.03452
		.03455
		.03452
R. W. Thatcher, Pullman, Wash.....	July 9.....	.03683
		.03684
		.03696
C. C. McDonnell, Bureau of Chemistry.....	July 20.....	.03536
		.03583
		.03575
Average.....		.03568

The results are all very close, the greatest difference being only 0.25 per cent. This method has also given satisfactory results in past years.

In view of the fact that this report was not presented at the meeting of the association, no recommendations will be made at this time.

**PRESIDENT SNYDER'S ADDRESS: THE TRAINING OF THE AGRICULTURAL CHEMIST.**

I have selected as the subject of the president's address for this, the twenty-fifth annual convention of the Association of Official Agricultural Chemists, "The Training of the Agricultural Chemist."

Any society or organization in order to be effectual and progressive must look well to its membership. Our society has been most fortunate in this respect, and it is to be hoped its ranks will continue to be filled with the same class of earnest, energetic workers as are here to-day. During the past quarter of a century this organization has accomplished most excellent results. I believe, however, that it has only entered upon its career of usefulness. Much credit is due to the founders for the high ideals of the association and for the cultivation of the true scientific spirit. Many of them

received their training in the great European laboratories, where they were students of Liebig, Fresenius, Voit, Hoffman, and Pasteur, and they have planted in this country the seed of true agricultural research. Most of the older members have relinquished their labors, and the work of the society may now be said to be in the hands of the second generation, who, it is hoped, will meet with as much success and foster the same spirit and ideals.

Originally agricultural chemists were in a way self-educated. They secured what knowledge they could of general and analytical chemistry and then applied it to the solution of agricultural problems. Naturally the work was largely analytical. "What does this substance contain?" was and is to-day the quest of the chemist. During the past few years, however, the domains of agricultural chemistry have been greatly enlarged and there is probably now no other branch of chemistry that calls for so wide a training. Organic, inorganic, industrial, physical, physiological, and sanitary chemists have definite channels within which their activities are confined, while the agricultural chemist must necessarily include in his domain a large portion of all of these. In dealing with the soil an extended knowledge of both inorganic and organic chemistry as well as of physical chemistry is requisite. Our knowledge of soils is necessarily much restricted because the chemistry of the silicates is so imperfectly understood, and so in the analysis of plant and animal substances and the interpretation of the results our knowledge is likewise very limited. While the data gained from the analysis of foodstuffs is exceedingly valuable, I do not believe that it is as much so as it is destined to be, and while chemistry is one of the most useful of the sciences in the study of agricultural problems, it is capable of being made still more valuable and useful.

One of the chief functions of the agricultural chemist is to correctly analyze agricultural products. In order to do this methods of analysis based upon rational principles must be devised, and this is one of the principal features of the work of this association. It is scarcely necessary for me to dwell upon its importance. Correct methods of analysis are essential, as without these chemistry would be entitled to no higher rank than alchemy. I do not believe that the importance of the development of correct methods for the analysis of agricultural products is as fully appreciated by experiment station workers as it should be. A large amount of the work that has been done is destined to be discredited and discarded because of errors in methods employed. Some of our experiment stations have been too impatient to secure immediate results and have not paid sufficient attention to methods of investigation. The study of the methods for analysis of foods and agricultural products can well be continued as the most prominent feature of this organization.

With the advance that is being made in general science and the greater recognition given agriculture, more extended provision should be made for the education and training of the prospective agricultural chemist. There are many institutions that offer excellent four-year courses in chemical engineering and other branches of chemistry leading to degrees. I know of no American institution where such a course is given in agricultural chemistry. Has not the time arrived for the establishment, in some of our institutions of courses of study having for their object the training of agricultural chemists? Certainly the importance and magnitude of the field would suggest the need of such courses. I shall not discuss the subjects that could most consistently form a part of the curriculum, but there should be a correlation of the different sciences blended with general and technical chemistry. As matters now stand, it is generally necessary for an experiment station to secure as assistants young chemists who have had but little training in analytical chemistry and give them special training in agricultural analysis. The experiment stations have to train their own assistants and by the time they have become reasonably proficient another institution or some industry offers a higher salary and then new assistants must be initiated, the process in some cases being repeated several times a year. Our

research work suffers because of this condition. Experiments are undertaken with one corps of assistants, a part of the work is done by another, and if the investigation is completed at all it is after many changes have been made. If some of our larger institutions would furnish more extended training in agricultural chemistry and better remuneration were given assistants so as to retain their services, conditions would be greatly improved. I do not consider that this lack of training of assistants is necessarily the fault of agricultural colleges, as their courses of study have been formulated with other objects in view than the training of scientists for research work. There are many interesting problems in agricultural chemistry which await investigation, and their correct solution would be of great benefit to mankind. The field of research is so large that this association can consistently encourage a larger number of workers.

In addition to the special technical training the agricultural chemist needs broad equipment in other lines so that he may be able to inaugurate useful lines of research and properly interpret his results. There are many chemists who are capable of making accurate and rapid analyses and prosecuting routine work, but are unable to outline an investigation, plan intricate details, carry the work to a satisfactory conclusion, and correctly interpret the results. There need be no fear of overcrowding in the realm of agricultural chemistry or necessity for forming a trade union to regulate the number practicing the profession. In this connection it is pleasing to note the greater recognition that is being given the agricultural chemist. About a decade ago the number of positions in this line were limited and the compensation exceedingly small. While neither the number of positions nor the compensation is now particularly large there has certainly been a material increase in both. For example, in the Department of Agriculture in 1897 the maximum salary paid was \$2,500 per year and the average to 12 chemists was \$1,541, while in 1907 the maximum salary was considerably greater and 47 chemists received an average of nearly \$2,000. On the whole, however, these salaries are smaller than are paid in many of the large educational institutions, although the rate of increase during the past ten years has been greater than in educational institutions, and if this continues the agricultural chemist bids fair in the near future to receive as large a compensation as workers in other lines of science. Much credit is due to our present Secretary of Agriculture for recognizing the importance of agricultural research and having the courage to advocate and recommend to Congress suitable compensation for agricultural scientists.

The position of the agricultural chemist in both the educational and business world is undergoing transition. He is being regarded as a greater factor in human and industrial progress than heretofore and I believe that with each decade he may reasonably expect greater opportunity to do good work, coupled with better compensation. Agricultural chemists have as a rule been underpaid; neither have they been given sufficient funds with which to prosecute their labors. In many laboratories bookshelves are not filled as they should be and makeshift apparatus is employed where better results could be secured if the chemist had at his command the literature covering the work of others upon the subject which he is investigating, and suitable apparatus and means for his work. There has been many a scientific surrender because of lack of funds for effectually carrying on the work.

As a nation we have taken great pride in the progress made by our industries, an advance more rapid than that of any other country. This in a large measure has been due to the work of the American chemist. There is scarcely an important industry but employs a well-trained chemist and has a suitably equipped testing laboratory. The steel, sugar, cement, and other great industries are practically applied chemistry. It has been said that the American chemist has contributed less than his quota to the advancement of science; he has, however, contributed his full share to the advancement of our industries. Instead of being a devotee of pure science he has advanced the domains of applied science. The agricultural chemist should

concern himself not only with the economic production of foodstuffs but should extend his work along the lines of their preparation and utilization. The production of food, while a very large and important subject, has associated with it its proper manufacture and utilization. The agricultural chemist should take a broader view than that of mere critic of the industries; and there is some danger when working along special food lines of his becoming too narrow in his consideration of the questions that present themselves. While adulteration and sophistication in any form should not be tolerated by the chemist, he should first make sure that it is adulteration, and in this connection there are destined to arise questions upon which scientific men materially and honestly differ. I should not care to see all scientists agree on all questions, as this would be detrimental to progress. There must be some attrition, and when differences arise they should be met in the true scientific spirit, each one being sure that the data and facts which he presents are absolutely correct in every detail. I believe the province of the chemist is first doing accurate analytical work. The stand which has been taken by this association is a most excellent one: That the meetings shall be open for discussion, that we invite thorough discussion of all subjects relating to the analysis of our agricultural products and the interpretation of their results, but that the views expressed by any one individual are not necessarily the official views of the association. In controversial questions it is well for the society to be conservative. We all recall the attempt of the French Academy of Science to settle the much-vexed question of atmospheric nitrogen as a source of plant food, and how, after examining the conflicting reports of Ville and Boussingault, the learned committee of the society reported that M. Ville's conclusions and results were consistent with his experiments. We well know how the conflicting work of these two investigators was later harmonized, and while the society attempted to decide the question the real question was not settled until years later. The best service this society can render the cause of agriculture is to continue along the lines followed by the founders, to improve the methods of analysis so that the work done by the official methods of the Association of Official Agricultural Chemists will command respect wherever quoted.

The food chemist should make a more careful and extended study of processes employed in the manufacture of foods. A purely theoretical knowledge of manufacturing processes may lead to erroneous conclusions. Some manufacturers of foods are doing more in the way of scientific investigation than are many of our universities and experiment stations. The encouragement given by the industries for the investigation of scientific subjects has been productive of fruitful results. Pasteur's classical work on fermentation was made possible by his connection with the industries. The agricultural chemist needs the help and assistance of the technical chemist.

One of our great needs is more funds with which to prosecute scientific inquiry. Men of science have the zeal and ability, but often fail for lack of funds to procure and construct scientific apparatus. And too often men in our universities who are specially adapted by nature for the prosecution of scientific investigations are overburdened with elementary instruction that could be more efficiently done by others. Many scientists attempt to do too much, and the result is a dissipation of energy.

Scientific work often suffers, too, because of the natural modesty of scientists, and sometimes those who accomplish the least but make the most noise, secure the lion's share of the funds for carrying on work. Some pseudo-scientists resort to cheap advertising that can not be too severely condemned. The best advertising a scientist can do is the publication of high-grade scientific work. It is a slow but a sure way of building up a permanent reputation. A scientist who maintains a press agency is not destined to make a permanent record.

Often science languishes because those immediately in authority are not sufficiently educated or naturally liberal enough to appreciate her claims or able to give wise and

intelligent direction to scientific investigations. Science should be completely segregated from politics as it is sometimes practiced, and she should not be dependent for her existence upon the whims of the spoilsman.

Science seeks to determine the truth. True science will not tolerate a falsehood nor perpetrate a fraud, and there is no place for the drone in the ranks of science; there have been a few who have made some progress by conjuring with scientific terms, looking wise, cultivating society, and catering to the whims of those in temporary authority and neglecting science. Others have had a brief but precarious existence as scientific pirates, appropriating to themselves the work and results of others, sometimes of advanced students and underpaid and dependent assistants. All true teachers and investigators enjoy having their assistants and students do good work and secure noteworthy results. A true scientist can honestly rejoice at seeing his colleague or coworker make a discovery. Petty jealousies are unworthy of science.

Agricultural chemistry is a great constructive agency and wealth producer. We are building our science for review by future generations. Let us build it well so as not to be ashamed of the workmanship. The true scientist bequeaths to mankind an invaluable legacy. Let us cultivate true science and not false ideals.

The proposed changes in the constitution, which had been made special order following the president's address, were considered. These changes were as follows:

In article 1, first sentence, substitute for the words "the United States," the words "North America."

In article 2, first sentence, second and third lines, insert the word "provincial" after the word "State;" also, in the third line of this sentence insert after the word "body" the phrase "in North America."

After discussion, the amendments were put to a vote and were carried.

A motion was made by Mr. Wiley to the effect that a referee and an associate referee on water analysis be appointed to study mineral, sanitary, irrigation, and technical waters, inasmuch as, under the food and drugs act, standard methods for potable waters were needed as well as for foods, while the analysis of irrigation waters was a purely agricultural question and also needed study and elaboration.

The motion that such referees be appointed was carried.

## FRIDAY—AFTERNOON SESSION.

### REPORT ON SOILS.

By S. D. AVERITT, *Referee.*

The association at its last meeting made only two recommendations affecting the referee's work this year.

- (1) That the modified J. L. Smith method for total potassium be further tested.
- (2) That the sodium peroxid fusion method for total phosphorus be adopted as a provisional method of this association and be further tested.

Including this year, these methods have been before the association three years, and it seemed very desirable to place before the association work sufficient in quantity and of such a quality as to enable it to dispose of them. From his experience as



a cooperater in the past, the referee thought it best not to ask for too much work, and in accordance with this view only one other line of investigation was requested of those who expressed a willingness to cooperate. In the opinion of the referee, the sodium peroxid fusion method for total phosphorus had some very serious disadvantages, particularly as to manipulation and length of time required for the determination. It was thought desirable to ask that a method in use in this laboratory be tested with a view to proposing it as a provisional method for total phosphorus in soils.

This method, which may be known as the magnesium nitrate method, is easy of manipulation and rapid, and in this laboratory has given uniformly as good results as the sodium peroxid fusion. Accordingly, it was asked that these two methods be compared.

Two well-known Kentucky soils were selected for this work. No. 1 is a cultivated soil from the western coal field. A complete analysis made by this station shows it to be poor in phosphates, organic matter, and nitrogen. No. 2 is a virgin soil from the Devonian in the eastern part of Clark County, known by analysis, as in the case of No. 1, to be particularly rich in phosphates, organic matter, and potash.

Samples were prepared and sent to fourteen chemists, who volunteered to aid in this work, with the following instructions:

#### INSTRUCTIONS.

(a) Make a determination of moisture by the official method, reporting the percentage.

(b) Weigh 10 grams of sodium peroxid into an iron or porcelain crucible and thoroughly mix with it 5 grams of the soil. If the soil is very low in organic matter, add a little starch to hasten the action. Heat the mixture carefully by applying the flame of a Bunsen burner directly upon the surface of the charge and the sides of the crucible until the action starts. Cover crucible until the reaction is over, and keep at a low red heat for fifteen minutes; do not allow fusion to take place. By means of a large funnel and a stream of hot water transfer the charge to a 500 cc measuring flask. Acidify with hydrochloric acid and boil. Let cool and make up to the mark. If the action has taken place properly there should be no particles of undecomposed soil in the bottom of the flask. Allow the silica to settle and draw off 200 cc of the clear solution.

Precipitate the iron, alumina, and phosphorus with ammonium hydroxid; filter, wash, return the precipitate to the beaker with a stream of water, holding the funnel over the beaker, and dissolve the precipitate in hot hydrochloric acid, pouring the acid upon the filter to dissolve any precipitate remaining. Evaporate the solution and washings to complete dryness on the water bath. Take up with dilute hydrochloric acid, heating if necessary, and filter out the silica. Evaporate filtrate and washings to about 10 cc, add 2 cc of concentrated nitric acid, and just neutralize with ammonium hydroxid. Clear up with nitric acid, avoiding an excess. Heat from 40° to 50° on water bath, add 15 cc of molybdic solution, keeping at this temperature for from one to two hours. Let stand overnight, filter, and wash free of acid with 0.1 per cent solution of ammonium nitrate; finally, once or twice with cold water. Transfer filter to beaker and dissolve in standard potassium hydroxid (1 cc equal to 0.2 mg of phosphorus), titrate the excess of potassium hydroxid with standard nitric acid, using phenolphthalein as indicator.

(c) Weigh into a 50 cc porcelain dish 5 grams of soil. Moisten with 5 to 7 cc of magnesium nitrate solution (*(g)* p. 2, Bul. 107, Bureau of Chemistry). Bring to dryness on water bath, burn off the organic matter at low redness; when cool, moisten slightly with water, add 10 cc of concentrated hydrochloric acid, digest two hours on water bath, keeping the dish covered with a watch glass; stir up two or three times during digestion.

Make up to 250 cc, mix well and throw on a dry folded filter, pouring back on the filter till the solution runs through clear. Take aliquots corresponding to 2 or 4 grams (4 grams in No. 1, 2 grams in No. 2), depending upon the amount of phosphorus present. Bring to dryness, take up with hydrochloric acid and water, filtering over pump. Filtrate and washings should not exceed 30 or 40 cc. Make alkaline with ammonia, and dissolve the precipitate with concentrated nitric acid, using a slight excess. Add gradually, while shaking, 5 to 15 cc molybdate solution (p. 2, Bul. 107). After standing a minute or two add 15 cc of ammonium nitrate (p. 2,

Bul. 107), shaking thoroughly. The solution should be kept at 40° or 50° C. for an hour, then let stand overnight at the room temperature, filter, and wash well with cold water. A Hirsch funnel with a double qualitative filter, S and S No. 597, cut to fit, and well pressed down around the edge with the finger after wetting and putting on pressure, is recommended. Put filter and precipitate back into the same flask, using as little water as possible for washing back into flask. Determine phosphorus volumetrically, using standard potassium hydroxid and nitric acid.

(d) Fuse 1 gram of soil according to the well known J. Lawrence Smith method. Transfer the fused mass to a porcelain dish, slake with hot water, grind finely with an agate pestle, and transfer to a filter. After washing free of chlorids, concentrate the filtrate and washings in a Jena beaker to about 20 cc, and filter. Slightly acidify the filtrate and washings with hydrochloric acid, concentrate in a platinum dish, add 1.5 cc of a platinic chlorid solution (10 cc contains 1 gram of platinum). Evaporate to a sirupy consistency, as usual, and wash with 80 per cent alcohol and ammonium chlorid solution.

(e) Determine potassium according to the regular J. Lawrence Smith method.<sup>a</sup>

The referee inclosed with his instructions to those who expressed a willingness to cooperate in the work a short personal letter, and had hoped that each one would contribute something to this report, but unfortunately, as it often happens, many were not able to send in results in time for use. The referee desires to express his thanks to the following chemists who have aided him in the work: A. W. Gregory, for the Illinois station; W. P. Kelly, for the Hawaii station; P. E. Brown, for the New Jersey station; G. S. Fraps, for the Texas station; W. B. Ellett, for the Virginia station; I. O. Schaub, for the Iowa station; and P. F. Trowbridge, for the Missouri station.

It will be seen from Table 1 that the results obtained by the sodium peroxid fusion and the magnesium nitrate method show practically no difference. One chemist gets results in Soil II somewhat higher than the others, but the amounts of phosphorus obtained by the two methods on three determinations are almost identical. In Soil I, with the exception of one determination, the maximum and minimum results are not bad duplicates.

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<sup>a</sup> Fresenius's Quantitative Chemical Analysis, p. 426.

TABLE 1.—Comparison of sodium peroxid fusion and magnesium nitrate methods for total phosphorus.

[Water-free basis.]

Analyst.	Soil No. I.		Soil No. II.	
	Sodium peroxid.	Magnesium nitrate.	Sodium peroxid.	Magnesium nitrate.
A. W. Gregory, Illinois.....	<i>Per cent.</i> 0.029 .028	<i>Per cent.</i> 0.027 .027	<i>Per cent.</i> 0.211 .210	<i>Per cent.</i> 0.224 .226
Average.....	.029	.027	.211	.225
W. P. Kelley, Hawaii.....		.030 .030 .031		.240 .244 .236
Average.....		.030		.240
P. E. Brown, New Jersey.....	.021 .029 .025 .025	.025 .025 .029	.281 .281 .285	.281 .285 .285
Average.....	.025	.026	.282	.284
E. C. Carlyle, Texas.....		.025 .023		.197 .201
Average.....		.024		.199
I. O. Schaub, Iowa <sup>a</sup> .....		.025		.213
W. B. Ellett, Virginia.....	.025 .024	.027 .028	.231 .230	.232 .232
Average.....	.025	.027	.231	.232
S. D. Averitt, Kentucky.....	.031 .032	.030 .030	.220 .213	.227 .229
Average.....	.031	.030	.217	.228
P. F. Trowbridge, Missouri <sup>a</sup> .....	.030	.029	.242	.219
General average.....	.028	.028	.237	.230

<sup>a</sup> Duplicates not reported.

The referee did not ask that these methods be checked by the carbonate fusion or standard method, as it is sometimes called, his work with the method having shown that it has the disadvantage of a large amount of soluble silica, necessitating dehydration, and in the case of a soil with any considerable amount of organic matter there exist favorable conditions for reduction and, consequently, some loss of phosphorus.

In Table 2 will be found the results of the referee's determination of total phosphorus in the two soils, working as follows: Making a carbonate fusion, then proceeding as for an accurate determination of silica, the silica evaporated with hydrofluoric acid, the residue taken up with hot, strong hydrochloric acid and added to the filtrate from the silica. Iron, alumina, and phosphorus precipitated with ammonium hydroxid, washed, redissolved, and the phosphorus determined volumetrically, as in the magnesium nitrate method.

It will be seen from the table that in Soil I, containing very little organic matter, the results compare very favorably with the other methods, but in Soil II, rich in organic matter, the average is lower.

TABLE 2.—*Duplicate determinations of phosphorus by carbonate fusion.*

[Water-free basis.]

Soil I.	Soil II.
<i>Per cent.</i>	<i>Per cent.</i>
0.025	0.202
.033	.220
.026	.207
-----	.207
.028	.209

In Table 3 will be found duplicate determinations of total phosphorus in soils by the magnesium nitrate digestion. Of these, Nos. 1127, 1202, and 1204 are presumably Texas soils, and the determinations were made by Mr. E. C. Carlyle, of the Texas station. The others are low phosphate soils of western Kentucky and were made as checks in the general work of this laboratory.

TABLE 3.—*Duplicate determinations of phosphorus by magnesium nitrate method.*

[Water-free basis.]

Soil No.	Determination 1.	Determination 2.	Soil No.	Determination 1.	Determination 2.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>
13	0.030	0.027	120	0.034	0.034
14	.051	.052	600	.035	.035
15	.046	.040	601	.043	.039
16	.052	.053	602	.028	.026
117	.046	.045	1127	.029	.027
118	.045	.045	1202	.031	.032
119	.034	.034	1204	.020	.021

Table 4 shows the modified method to compare very favorably with the regular J. Lawrence Smith method for total potassium, the former method giving in the general average 0.01 per cent more potassium in both soils. Taking into consideration the fact that the work is done on 1 gram the agreement is as close as could be expected. Referring to the work done by these methods in 1906 and 1907, it will be seen that the results are in the main concordant.

TABLE 4.—Comparison of modified and Smith methods for total potassium.

[Water-free basis.]

Analyst.	Soil I.		Soil II.	
	Modified.	Smith.	Modified.	Smith.
A. W. Gregory, Illinois.....	<i>Per cent.</i> 1.156 1.157 1.182 1.156 1.158	<i>Per cent.</i> 1.205 1.175 1.164 1.208 1.223	<i>Per cent.</i> 1.568 1.547 1.566 1.552 1.547	<i>Per cent.</i> 1.582 1.598 1.594 1.586 1.566
Average.....	1.162	1.195	1.556	1.585
W. B. Ellett, Virginia.....	1.068 1.081	1.183 1.121	1.495 1.487	1.564 1.536
Average.....	1.075	1.152	1.491	1.550
S. D. Averitt, Kentucky.....	1.190 1.185 1.214	1.162 1.170 1.202	1.552 1.546 1.531	1.491 1.541 1.565
Average.....	1.196	1.178	1.543	1.532
P. F. Trowbridge, Missouri <sup>a</sup> .....	1.177	1.186	1.512	<sup>c</sup> 1.357
A. A. Wells, Iowa.....	1.271 1.275	1.121 1.134	1.608 1.702	1.751 1.760
Average.....	1.273	1.127	1.655	<sup>c</sup> 1.755
O. M. Shedd, Kentucky <sup>b</sup> .....				1.511 1.627
Average.....				1.569
I. O. Schaub, Iowa.....	1.320 1.440	..... .....	1.656 1.678	..... .....
Average.....	<sup>c</sup> 1.380	.....	1.667	.....
General average.....	1.177	1.168	1.571	1.556

<sup>a</sup> Duplicates not reported.<sup>b</sup> Too late to be included in the general average.<sup>c</sup> Not included in the general average.

## COMMENTS OF ANALYSTS.

*P. E. Brown:* The magnesium nitrate method is undoubtedly quicker and easier of manipulation than the peroxid fusion method. It has the advantage that there is not nearly such a large amount of silica to get rid of, as it was found necessary to dehydrate three or four times with the peroxid fusion method. Then, too, in the first operation of the fusion method there seems to be an uncertainty of reaction while avoiding fusion, which is of course eliminated in the other method.

You will notice from the results that the agreement is fair with a tendency for the new method to give slightly higher results. However, if the first determination in Soil I by the fusion method is eliminated, the agreement is much better. On the whole the magnesium nitrate method seems to me to be undoubtedly superior to the other.

*W. P. Kelley:* I find the magnesium nitrate method as outlined by you to be a very simple and convenient scheme for determining the phosphoric acid in soils; and while I have not had an opportunity to compare this method with others, I have no doubt that the results are reliable.

## RECOMMENDATIONS.

The work done this year, while not as extensive as the referee had wished, still warrants in his opinion three conclusions, especially when it is remembered that work along the same line last year and the year before is mainly concordant in the matter of results: First, that the modified J. Lawrence Smith method for total potassium compares very favorably with the regular method and is somewhat shorter; second,

that the sodium peroxid fusion method for total phosphorus gives good results, but the manipulation presents some difficulty, and the time required for making the determinations is a disadvantage; third, that the magnesium nitrate method gives uniformly as good results for total phosphorus as the sodium peroxid fusion, and is quick and easy of manipulation. With these facts in view, the referee would make the following recommendations:

- (1) That the modified J. L. Smith method for total potassium be adopted as an optional method of this association.
- (2) That the sodium peroxid fusion for total phosphorus be adopted as an official method.
- (3) That the magnesium nitrate method for total phosphorus be adopted as a provisional method of this association and be further tested.

## REPORT ON THE DETERMINATION OF CALCIUM CARBONATE IN SOILS.

By JACOB G. LIPMAN, *Associate Referee.*

Systematic determinations of calcium carbonate in cultivated soils seem highly desirable in view of its important functions in crop production. Unfortunately, there is no unanimity of opinion among chemists as to the methods best adapted for this work. When the proportion of calcium and magnesium carbonates exceeds 1 per cent, fairly accurate determinations may be made by the liberation of carbon dioxide and its absorption and weighing in potash solutions. But when the proportion of carbonate is small, as is true of so many of our soils, the quantity of carbon dioxide which remains in solution in the acid is very large in proportion to its entire amount. This source of error has frequently been commented upon and has led to several more or less successful attempts to correct it.<sup>a</sup>

The associate referee on soils thought it advisable, therefore, to outline some cooperative work on one or two promising methods for the determination of carbonates in soils. Samples of two different soils were sent to eleven members of the association who had signified their willingness to cooperate in the testing of soil-analytical methods. It was suggested that determinations of carbonates be made in the samples by Knorr's method as described in Wiley's *Agricultural Analysis*.<sup>b</sup> Where possible, the results secured by Knorr's method were to be checked by the method described by Amos in the *Journal of Agricultural Science*.<sup>c</sup>

The samples were sent out early in September, and analyses were made and reported by W. B. Ellett of the Virginia station, by Percy E. Brown of the New Jersey station, and Ernest Van Alstine of the Illinois station. Mr. Van Alstine's data were transmitted to the associate referee by Mr. Hopkins.

Mr. Ellett and Mr. Brown used Knorr's apparatus for the determination of the carbon dioxide. Mr. Van Alstine employed the method regularly used at the Illinois station and consisting of the liberation of the carbon dioxide "by boiling with hydrochloric acid and ascertaining the quantity of carbon dioxide evolved by measuring before and after absorption by a caustic potash solution." The results were as follows:

<sup>a</sup> See Hall and Russel, *A Method for Determining Small Quantities of Carbonates*, *Transactions, J. Chem. Soc., London, 1902, 81:81.*

<sup>b</sup> Vol. 1, p. 338.

<sup>c</sup> 1905, 1:322.

*Determination of carbon dioxid in soils.*

[Percentage of dry soil.]

Analyst.	Soil No. 1.	Soil No. 2.
E. Van Alstine, Illinois.....	0.025	0.020
	.025	.020
	.027	.020
	.024	.020
Average.....	.025	.020
W. B. Ellett, Virginia.....	.034	.022
	.030	.025
Average.....	.032	.023
P. E. Brown, New Jersey.....	.031	.022
	.034	.026
	.030	.021
Average.....	.032	.023

The results submitted by Ellett and Brown agree very satisfactorily. Those submitted by Van Alstine are markedly lower, especially in the case of soil No. 2. Apparently the amount of carbon dioxid which remained in solution in the latter work is the cause of the lower results. Evidently Knorr's apparatus is efficient for the determination of comparatively slight amounts of carbonates; however, it is desirable that further work be done along this line, and the associate referee would therefore recommend that it be continued with certain modifications for at least another year.

**REPORT ON POTASH.**By B. B. Ross, *Referee.*

The work on potash for the past year has included cooperative tests of the regular official method in comparison with the phosphomolybdic volumetric method, and, in addition, the referee, associate referee, and some cooperating chemists have made comparative tests with some special methods which will be described in the latter portion of this report.

Twenty laboratories expressed a desire to take part in the cooperative work on potash samples, but reports were received from only eight laboratories.

Two samples were sent out for analysis to each laboratory taking part in the work, sample No. 1 being high-grade commercial sulphate of potash, while sample No. 2 was a mixed fertilizer, the ingredients of which were acid phosphate, cottonseed meal, dried blood, potassium chlorid, and a small amount of magnesium sulphate.

The following instructions with regard to the work were sent out to all cooperating chemists, the details of the volumetric method being those given by the referee for 1906 and 1907, Mr. A. L. Knisely, who had given much time and attention to a study of the phosphomolybdic method.

**OUTLINE OF ASSOCIATION POTASH WORK.**

Sample No. 1. Commercial sulphate of potash.

Sample No. 2. A complete mixed fertilizer, the nitrogen of which is derived from cottonseed meal and dried blood.

Potash in these samples should be determined both by the official method and the proposed volumetric method involving use of phosphomolybdic acid.

*Reagents.*

*Nitric acid.*—50 cc of nitric acid (1.40 sp. gr.) in 1,000 cc of water.

*Sodium nitrate wash.*—10 grams of sodium nitrate per 1,000 cc of water.

*Phosphomolybdic acid solution.*—100 grams of phosphomolybdic acid (Kahlbaum's preferred) in 750 cc of water and 250 cc of nitric acid (1.40 sp. gr.). This solution must be freshly prepared—not over three or four days old before using. If properly made the evaporated residue from a portion of this solution is never white and readily redissolves in the dilute nitric acid solution *in the cold*.

*Standard solutions.*—Standard caustic potash and nitric acid prepared for volumetric phosphoric acid diluted to 2 volumes. One cubic centimeter of this potassium hydroxid solution is equal to 0.812 mg of potassium oxid.

*Determination.*

Transfer 10 cc of solution to a platinum dish, add 0.25 cc of sulphuric acid (1 to 1). Evaporate to dryness and ignite to whiteness. Dissolve residue in hot water plus a few drops of hydrochloric acid and transfer to a tall 200 cc beaker, add 30 cc phosphomolybdic acid solution and slowly evaporate to complete dryness on top of a steam bath.

It requires approximately 22 mg of phosphomolybdic acid, in order to have an excess, for each milligram of potassium oxid present.

Add 30 cc of nitric acid wash to the dried residue and stir thoroughly in the cold, with a grinding motion with a policeman, allow to settle a moment and decant supernatant liquid at once through a gooch crucible packed with moist filter paper pulp, approximately one-sixteenth inch in thickness. Wash twice by decantation with sodium nitrate wash, transfer precipitate to a gooch and wash with sodium nitrate wash until acid free. Transfer gooch to casserole, run in excess standard alkali solution and add phenolphthalein. Heat to boiling and titrate excess alkali with standard acid.

Some samples of asbestos seem to hold or "fix" some of the excess acid, making the gooch filter very hard to wash acid free. Hence it is suggested to use a paper pulp filter. It is also desirable to make comparative tests, employing the usual asbestos filter.

If excess of phosphomolybdic acid has been used, the dried residue has a reddish hue. If excess has not been added the residue is bright yellow. Residue should not appear white.

In each case, run blanks to ascertain corrections to be made for impurities.

It is also desired that in sample No. 1 determinations of potash be made, not only by the official method (which provides for direct evaporation of the solution without addition of ammonia and ammonium oxalate), but also by the method applicable to mixed fertilizers, adding ammonia and ammonium oxalate, followed by evaporation and subsequent ignition with sulphuric acid.

Several chemists have urged that this latter method of procedure be tried, as it is claimed that the official method for potash salts gives too high results owing to impure precipitates.

The reports of results of cooperating chemists are as follows:



## Potash results reported by cooperating chemists.

Analyst.	Sample No. 1.			Sample No. 2.	
	Official method.	Volumetric method.	Official method plus ammonia and ammonium oxalate.	Official method.	Volumetric method.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
E. L. Baker, Geneva, N. Y. ....	51.32 51.24	51.26 51.30	50.16 50.16	4.41 4.43	4.89 4.89 4.72
S. E. Asbury, College Station, Tex. ....	51.40		50.66	4.42	
E. C. Carlyle, College Station, Tex. ....					4.65 4.40
G. Farnham, Cincinnati, Ohio. ....	50.60		49.81	4.44	
G. Farnham, Cincinnati, Ohio. ....	50.56		49.77	4.40	
J. H. Mitchell, Clemson College, S. C. ....	50.80	50.41	50.64	4.44	4.45
B. F. Robertson, Clemson College, S. C. ....	50.75	50.60	50.62	4.47	4.49
G. T. Beyer, Chicago, Ill. ....	50.71	50.09	50.22	4.31	4.20
Laboratory of Armour & Co. ....	50.82 50.84	50.61 50.09	50.46 50.55	4.35 4.34	4.45
O. M. Shedd, Lexington, Ky. ....	50.68 50.73 a 50.29 a 50.43		50.16 50.08	4.38 4.43	
H. E. Taylor, Chicago, Ill. ....	51.02	58.19	50.28	4.42	4.66 4.68
Laboratory of Swift & Co. ....		61.60 58.00 57.96			5.01 5.14 4.99
C. R. Bragdon, Chicago, Ill. ....		59.62			
Laboratory of Swift & Co. ....		58.80		4.43	4.90
C. L. Hare, Auburn, Ala. ....		59.11			4.69 4.30 4.60
A. M. Ransom and T. Bragg, Auburn, Ala. ....	51.20 51.36	51.96 52.60	49.80 50.10	4.44 4.38	
Average. ....	50.87		50.23	4.40	
M. G. Donk, Washington, D. C. <sup>b</sup> ....	50.92 50.80		49.08 49.00	4.38 4.35	4.21 4.32 4.41 4.36

<sup>a</sup> 0.1 gram used.

<sup>b</sup> Results received too late to be included in the averages.

## COMMENTS BY ANALYSTS.

*E. L. Baker, Geneva, N. Y.:* Moist filter paper pulp was used in one of each set of duplicates and a thick pad of asbestos in the other, with no appreciable variation in results. In some cases the precipitate showed a tendency to run through the filter paper pulp. It was easier, however, to wash the filter paper free from acid. Corrections were made for a blank of 0.3 cc of potassium hydroxid. Corrections were also made for blanks in the official method. You will notice that in the case of the mixed fertilizer the two methods differ by about 0.4 of a per cent. During a series of determinations I was unable to obtain any closer agreement.

*E. C. Carlyle, College Station, Tex.:* The use of pulped filter paper for filtering the phosphomolybdate is found satisfactory and it reduces the bumping when the liquid is heated for the purpose of dissolving the potash salt.

*G. S. Farnham, Cincinnati, Ohio:* I regret to report that I failed to get checks for the volumetric method.

*P. Rudnick, Chicago, Ill.:* It seems from the results by the official method that there is some truth in the claim that the method for mixed fertilizers when applied to sulphate of potash gives somewhat lower results. The proposed volumetric method

was given as thorough a trial as time and opportunity permitted, and although the results obtained were not very satisfactory, the method itself certainly looks very promising. The difficulties are, first, the very small amount of sample taken; second, the extreme proneness of the precipitate to go through the filter; third, the great difficulty of removing the precipitate from the sides of the beaker or casserole; fourth, the difficulty in washing all the nitric acid out of the precipitate. Asbestos is much inferior to paper pulp for filtering.

*W. D. Richardson, Chicago, Ill.:* With the volumetric method, following your directions, we did not have very good success.

*O. M. Shedd, Lexington, Ky.:* From my work, I would suggest on samples similar to No. 1 that ammonium hydroxid and ammonium oxalate be added as in the case of mixed fertilizers, and that an aliquot be used of 0.10 to 0.20 gram and not over 0.25 gram, instead of 0.50 gram, for the smaller potassium platonic chlorid precipitate can be worked better; besides it is my experience that very large precipitates carry down a greater proportion of impurities.

In addition to the above results Mr. Shedd made determinations in sample No. 1 by evaporating with sulphuric acid, igniting, and then evaporating with platonic chlorid. The results secured were 49.75 and 49.88 per cent.

*M. G. Donk, Washington, D. C.:* Could get no satisfactory results on sample No. 1 by the volumetric method.

E. L. Baker, associate referee, made additional comparative determinations of potash in several samples of potash salts, both with and without the use of ammonia and ammonium oxalate, in making up the solution, the results being as follows:

*Comparison of results obtained with and without the use of ammonia and ammonium oxalate.*

Sample.	With.	Without.	Sample.	With.	Without.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>
Kainit.....	12.94	13.13	Kainit.....	13.42	13.48
	12.95	13.18		13.30	13.52
Muriate and sulphate.....	49.68	51.20	Muriate.....	49.12	50.04
	49.64	51.16		49.14	49.84

Mr. Baker also reported results of determinations of potash in a number of samples by the sodium cobalti-nitrite method first proposed as a quantitative process by Adie and Wood.<sup>a</sup> This method involved the use of sodium cobalti-nitrite as a precipitant, and in the original process precipitation was effected in the presence of acetic acid in a solution which should contain from 0.5 to 1 per cent of potash. Drushel<sup>b</sup> has modified this method as follows:

The solution of a potassium salt, containing not more than 0.2 gram of potassium oxid and free from ammonium salt, was treated with a rather large excess of sodium cobalti-nitrite solution, acidified with acetic acid, and evaporated to a pasty condition over the steam bath. It was then cooled and treated with from 50 to 100 cc of cold water, and stirred until the excess of sodium cobalti-nitrite was dissolved. It was allowed to settle and was decanted through a perforated crucible fitted with an asbestos felt. The precipitate was washed two or three times by decantation, after which it was transferred to the crucible and thoroughly washed with cold water. In the meantime a measured excess of standard potassium permanganate was diluted to ten times its volume and heated nearly to boiling. Into this the precipitate and felt were transferred and stirred, after which the crucible was also put into the solution, since particles of the precipitate stick persistently to its sides. After the oxidation had proceeded five or six minutes manganese hydroxid separated out and the

<sup>a</sup> J. Chem. Soc., 77: 1076.

<sup>b</sup> Amer. J. Sci., 24: 433; Chem. News, 97: 124.

color of the solution darkened. At this point from 5 to 25 cc of sulphuric acid (1:2) were added, and the solution, after stirring, was allowed to stand a few minutes. Then a measured amount of standard oxalic acid, containing 50 cc of strong sulphuric acid per liter, was run in from a burette, taking care to add an excess. The temperature was maintained a little below the boiling point until the solution became colorless and the manganese hydroxid had completely dissolved. It was then titrated to color by permanganate in the usual manner. From the whole amount of permanganate employed, the permanganate equivalent of the oxalic acid used was subtracted and the remainder multiplied by the factor calculated for the strength of permanganate used, 0.000856 being the factor for strictly tenth-normal potassium permanganate.

While work was in progress in the referee's laboratory with a view to testing the adaptability of the cobalti-nitrite methods, letters were received from several cooperating chemists commending this process quite strongly, as a result of some preliminary work which had been done with it. Mr. A. M. Peter, of the Kentucky station, reported that by the cobalti-nitrite method results of 49.89 and 49.92 per cent were obtained by Mr. G. Edgar for sample No. 1, as against 49.82 by the official method, while for sample No. 2, 4.50 and 4.48 per cent of potash was found, as against 4.41 by the official method. Mr. Baker obtained the following results on the official samples by Drushel's modification: No. 1, 50.24 and 50.85 per cent; No. 2, 4.46 and 4.31 per cent. Following are results reported by Mr. Baker, using the original Adie and Wood method, in which precipitation is effected without evaporation:

*Comparison of potash determinations by the original Adie and Wood cobalti-nitrite method (volumetric) and the official method.*

Sample.	Official method.	Cobalti-nitrite method.	Sample.	Official method.	Cobalti-nitrite method.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>
Sulphate.....	53.54	54.08	Kainit a.....	13.13	10.14
	53.70	54.08		13.18	10.28
Muriate and sulphate.....	50.68	50.71	Mixed fertilizer a.....	4.95	4.21
	50.84	50.71		5.06	4.21
Mixed fertilizer a.....	10.41	10.78	Mixed fertilizer a.....	1.28	.40
	10.41	10.92		1.29	.40
Kainit a.....	13.48	10.64			
	13.52	10.71			

\* The kainits and mixed fertilizers evidently did not entirely precipitate owing, probably, to improper concentration.

#### CONCLUSIONS.

It appears from the result of this year's work, that while some good results are obtained by the volumetric method, there are difficulties connected with the working of the process which affect the reliability and rapidity of its execution. Among these may be mentioned the trouble experienced in washing the precipitate free of acid and the tendency of the precipitate to run through, while the smallness of the aliquot used in the determinations would, of course, tend to affect the accuracy of the results.

On this account it would seem desirable that work with this method be held in abeyance for the present and that a trial be made of the cobalti-nitrite method with a view to determining its adaptability to fertilizer work.

The results of tests of the employment of ammonia and ammonium oxalate in potash determinations in potash salts indicated that lower figures are secured in this way, so that from this partial investigation the contention of those who claim that the usual method gives impure precipitates would seem to be sustained. However, no positive conclusion can be reached from the limited data at hand and hence this question should be investigated further.

## REPORT OF COMMITTEE C (FOOD ADULTERATION).

By L. M. TOLMAN, *Chairman.*

## WINES.

It is recommended—

(1) That a committee of five be appointed to cooperate with the Bureau of Standards in drawing up a standard alcohol table.

Adopted.

(2) That the question of a standard temperature of 20° for specific gravity and alcohol determinations be also referred to the committee of five.

Adopted.

(3) That the following subjects be given further study:

(a) Methods for determining glycerol.

(b) Methods for determining total, fixed, and volatile acids.

(c) Methods for determining coloring matter in genuine wines.

Adopted.

## FLAVORING EXTRACTS.

It is recommended—

That the colorimetric method for the determination of citral in lemon extract be adopted as provisional. (See page 32.)

Adopted.

## DAIRY PRODUCTS.

It is recommended—

That the Baier and Neumann method for the detection of succinate of lime in milk and cream be studied. (See page 53.)

Adopted.

## DISTILLED LIQUORS.

It is recommended—

(1) That the modified Allen-Marquardt method for the determination of fusel oil be made a provisional method.

Adopted.

(2) That in the present method (Bul. 107, Rev., p. 98) a second washing with sodium sulphate be prescribed.

Adopted.

(3) That the method for the determination of water-insoluble color in whiskies be made provisional. (See page 207.)

Adopted.

(4) That the modified Marsh test for the quantitative determination of the color insoluble in amyl alcohol be adopted as a provisional method. (See page 206.)

Adopted.

(5) That the provisional Roesse method for determining fusel oil (Bul. 107, Rev., p. 97) be dropped.

Adopted.

## SPICES.

It is recommended—

That methods for the detection of added oil in paprika be further studied.

Adopted.

## MEAT AND FISH.

It is recommended—

(1) That the study be continued in an attempt to apply, improve, or devise methods for the most accurate separation possible of the various protein bodies in meat.

(2) That the method for determining ammoniacal nitrogen by distillation under reduced pressure be compared with the magnesium oxid method now generally used. (Bul. 107, p. 9.)

## CEREAL PRODUCTS.

It is recommended—

That methods applicable for the separation of the gluten constituents of flour be studied, tests to be made upon the several grades, as patents, first and second clears, and on flours produced from different varieties and types of wheat.

Adopted.

## CANNED VEGETABLES.

It is recommended—

That methods for the detection of soaked peas be further studied.

Adopted.

## MEAT PROTEIDS.

It is recommended—

That the work on the separation of meat proteids be continued along the lines pursued the past year.

Adopted.

## TEA, COFFEE, AND COCOA.

It is recommended—

(1) That methods for the estimation of caffein be further studied. (Original Gomborg Method, *J. Amer. Chem. Soc.*, 1896, p. 331, and modifications.)

Adopted.

(2) That the Dubois method for the determination of sugars in chocolate be further studied. (*J. Amer. Chem. Soc.*, 1907, 29: 556; *Bul.* 107, p. 256.)

(3) That the Doolittle and Woodruff method (*Bul.* 105, p. 48) for extract in tea be substituted for the Krauch method (*Bul.* 107, p. 149, sec. 5) as provisional.

Adopted.

## COLORS.

It is recommended—

(1) That an effort be made to obtain authentic samples of vegetable or natural coloring matters, such as are used in food products.

(2) That a study be made of the characteristics of vegetable coloring matters and methods of identification.

(3) That pure colors be synthetically prepared to serve as standards.

(4) That the separation and identification of mixed colors be studied.

These recommendations were adopted.

## REPORT OF COMMITTEE ON THE TESTING OF CHEMICAL REAGENTS.

By L. F. KEBLER, *Chairman.*

There has been a marked improvement in the chemical reagents examined by the chairman of the committee during the past year. This, however, may be largely due to a weeding-out process that has been going on for several years. It was a common experience a few years ago to be compelled to report adversely on the quality of many chemicals which included not only actual adulteration, but indicated gross carelessness in manufacturing and packing. The chemicals found to be of inferior quality during the past year were generally lacking in certain minor respects; for example, contamination with insoluble material or some associated impurity which would be detrimental to the analytical operations for which the reagent was to be employed.

One of the difficult features at present is a satisfactory nomenclature. In the past it has been common to use in connection with chemicals supposed to be of high quality the abbreviation C. P., but this abbreviation has come to be meaningless and should be discontinued. It still serves one good purpose and that is, if a chemical

is accompanied by this designation the chemist can reject it on general principles if found to be of unsatisfactory quality. Other specifications, such as pure, purissimum, reagent, commercial, etc., also have vague meanings which are used by manufacturers, dealers, and brokers, simply as a means for selling certain chemicals. The past year has seen a marked improvement along these lines, due largely to the instrumentality of the food and drugs act. The term "commercial" has been replaced largely by the term "technical" for the reason that the former name was vague and was used in connection with products which might be used for either food, drug, or technical purposes; for example, "sodium phosphate, commercial," did not give any information at all as to the quality of the product, and while the name would suggest that it was not of high grade, yet it was not uncommon for highly arsenical sodium phosphate to find its way into the drug trade, rather than to the boiler compound manufactory, and thus do harm. The terms pure, purissimum, and reagent are also gradually losing their standing, and the question arises, What form of nomenclature should be employed in order to obtain chemicals of the desired quality?

The chairman, therefore, recommends that the committee be instructed to investigate the question of nomenclature to be used in connection with chemical reagents and report at the next meeting.

The report was accepted and the recommendation made was approved by the association.

#### REPORT OF COMMITTEE ON FOOD STANDARDS.

On behalf of the food standards committee of the association, the chairman, Mr. Frear, submitted a detailed report of the work done by the joint committee on food standards during the year. This covers the adoption of tentative standards for manufactured meats, malt liquors, and spirituous liquors. The report of the committee was accepted by the association.

The president announced the following committee on the standardization of alcohol tables: L. M. Tolman, M. E. Jaffa, A. B. Adams, R. J. Davidson, H. E. Barnard.

#### REPORT OF COMMITTEE ON NOMINATIONS.

Mr. Davidson, as chairman of the committee on nominations, then presented the following report: For president, Mr. W. D. Bigelow; for vice-president, Mr. W. A. Withers; for secretary, Mr. H. W. Wiley; for additional members of the executive committee, Mr. E. F. Ladd and Mr. E. B. Holland.

The chairman of the committee was instructed to cast the unanimous vote of the association for the officers named.

On motion by Mr. Davidson the question of the amount of wash water to be employed in the treatment of the residue from the ammonium citrate digestion in the determination of phosphoric acid was referred to Committee A for recommendation.

## THE ASSAYING OF ALKALOIDAL DRUGS.

By C. E. PARKER.

The original drug assay methods of the last revision of the United States Pharmacopœia, on the whole, fairly represented the existing status of this branch of chemical analysis. They were formulated under the instruction of the convention for revising the Pharmacopœia that assay processes should be "reasonably simple (both as to methods and apparatus required) and lead to fairly uniform results in different hands."

The probability being somewhat vague that they would be made the basis for general legal regulation, a high degree of accuracy did not appear important, and similar moderate standards of requirement have possibly influenced the evolution of drug assay methods generally. After the passage of the federal food and drugs act of June 30, 1906, the committee on revision made a number of corrections and modifications in the text of the Pharmacopœia that it might better meet the new requirements.

Judged from the point of view of the official chemist and prospective expert witness before the courts, the cooperative work as far as it has gone has not shown that the pharmacopœial methods lead to fairly uniform results in different hands. This is probably due more to lack of detail in the instructions than to any fundamental defects in the methods. It is evident that losses occurring at certain stages in the processes may be prevented by suitable alterations in the methods, and that the unfavorable results on some drug samples may, to a considerable extent, be attributed to the powder not being of a proper fineness.

The samples sent out this year were from supplies ordered to be according to the United States Pharmacopœia, both as to assay and fineness of powder. The sample of belladonna root has been criticised as being a finer powder than specified by the Pharmacopœia, and, therefore, likely to give higher results and too favorable reports on the method. Other samples of drugs have been said to be too coarse, and, therefore, unfair to the methods. The point is well taken, but the only way to obtain a powder of exactly the pharmacopœial size would be to separate with suitable screens all larger and smaller particles produced by the mill, and such a product would not be representative of the original drug. The proper solution of the difficulty would seem to be the provision of suitable apparatus for grinding all drug samples for assay at least as fine as the Pharmacopœia requires and as much finer as experience shall show to be expedient.

The theoretical objections to the aliquot method of extraction may be justified when the grosser imperfections in the methods have been eliminated, but so far results fail to demonstrate the superior reliability of the total extraction method, and judgment must be suspended.

It was thought advisable to traverse again the ground covered last year when only three analysts participated, comprising methods for the assay of aconite root, belladonna leaves, belladonna root, cinchona bark (yellow and red), cocoa leaves, colchicum corm, and colchicum seeds. Samples of these drugs delivered as being of pharmacopœial quality and as ground to the fineness of powder specified in the respective pharmacopœial assay methods were supplied to all collaborators with the following directions, and instructions that all calculations and solutions except as otherwise specified be based on the data of the United States Pharmacopœia, eighth revision, with the additions and corrections dated May 1 and June 1, 1907.

The provisional methods appearing in Bulletin 107, revised, pages 258-259, were slightly modified in accordance with the experience of last year. *Only the modifications are reprinted below and the changes are italicized.*



## DETERMINATION OF ALKALOID.

*Total extraction method.*

Into a 200 cc flask weigh 10 grams of the powdered drug, add about 75 cc of ether-chloroform mixture (5 to 1 by volume), rotate and add 5 cc of 10 per cent ammonia water, cork, shake well and often during two hours. \* \* \*

*Aliquot method.*

Into a 200 cc flask weigh 15 grams of the powdered drug, add 150 cc of ether-chloroform mixture (5 to 1 by volume), cork and shake often for several minutes. Add 5 cc of ammonia water (10 per cent), shake frequently during two hours. Add 15 cc of water, or sufficient to agglomerate the drug, shake, let settle a few minutes, and then decant 100 cc of the clear solution into a graduated cylinder. \* \* \*

NOTE.—Under both methods substitute “a few cubic centimeters” for the words “a small portion,” referring to the ether-chloroform rinsing.

## CINCHONA BARK.

*Method I.*

United States Pharmacopœia VIII, page 102. Report total and ether-soluble alkaloids.

*Method II.*

Total extraction, gravimetric. *In extracting the drug let stand over night.*

The work on yellow and red cinchona and colchicum corm and root being quite incomplete is not included in this report. The instructions should be followed as strictly as possible, notes being taken during the work of any difficulties encountered, objections to the methods, necessary or advisable modifications with the reasons therefor, and any ambiguity or indefiniteness in the instructions should be indicated. The value of collaborators' reports is much enhanced by this practice. (See tabulation at close of report, p. 134.)

For comparing in respect to their variability the results obtained by the different methods from the several drugs, the average result for each method is taken as a basis, and the proportion of all the results approaching within 10 per cent above or below this average is given, and in addition the proportion approaching within 15 per cent of the average. Reserving the question of absolute accuracy, results commonly varying over a range of more than 20 per cent in different hands can scarcely be described as fairly uniform, nor can methods yielding such results be considered satisfactory for the purposes of the official chemist. Only one operator has reported any dissatisfaction with the behavior of cochineal as an indicator, though another has substituted hematoxilin for it throughout.

## DETAILS OF MANIPULATION.

The United States Pharmacopœia assay methods generally direct that the initial digestion of the drug with a solvent for the purpose of extracting the active principle be accompanied by an indefinite amount of agitation. In certain cases continuous agitation by means of suitable mechanism is alternatively directed, or preferred. The expression “frequent shaking” is susceptible of various interpretations, and it would be advisable to adopt the requirement of continuous agitation in all cases.

A number of collaborators reported difficulty in decanting 100 cc of the solvent mixture in extracting the drug by the aliquot method, and some were compelled to use forcible expression or continue the assay with less than 100 cc, computing the result on the basis of the aliquot part decanted. This occurred especially with belladonna leaves and cocoa leaves and is attributable to the coarseness of the samples. In the Drug Division it was found practicable to obtain 100 cc by decanting the mixture of drug and solvent as completely as possible into a small percolator provided



with a purified cotton plug in the neck, and loosely stoppering the same while the filtrate collected in a 100 cc flask. Excessive evaporation was thus avoided. With samples of a suitable degree of fineness 100 cc could be decanted without difficulty.

One worker filters the final solution of alkaloid in volatile solvent before evaporating off the latter. If the funnel be kept covered during filtration, and if the filter be properly washed, losses may be avoided and the alkaloid obtained in a cleaner condition than without filtration.

#### DISCUSSION OF RESULTS.

##### ACONITE ROOT.

This sample was delivered as No. 40 powder. The following proportions passed through the respective sieves:

	Grams.
No. 60.....	0
No. 50.....	7
No. 40.....	11
No. 20.....	82
Total.....	100

Most of the powder was therefore coarser than the Pharmacopœia directs for assay samples.

The three gravimetric results by Method I are too few in number to base upon them any conclusion. Only 32 per cent of the volumetric results by Method I (U. S. P.) come within 10 per cent of the average and only 59 per cent come within 15 per cent, and the results, both gravimetric and volumetric, by (II) are, on the whole, as bad or worse. The average results by the two methods are in very good agreement, but considerably under the United States Pharmacopœia standard of 0.50 per cent. It is quite possible that higher and more uniform results might have been obtained with a finely powdered sample.

On comparison of the corresponding gravimetric and volumetric results by (II) which we may assume were obtained by weighing and then titrating the same alkaloidal residues, it will be observed that in about one-half the instances the volumetric result is higher than the gravimetric, though it can not be assumed that these residues consist of absolutely pure alkaloid. The factor for aconitine (0.064) employed in computing the volumetric result is too high, and the residue contains alkaloidal matter of lower molecular weight than 640, resulting from the decomposition of aconitin. It is probable that the volumetric results by (I) are affected by a similar error. These considerations tend to support the contention of Doctor Lyons and others that chemical assays of aconite should be confirmed by the so-called "physiological test."

In Method, I Mr. Fuller considers the evaporation of the alcoholic percolate to dryness at a temperature not exceeding 60° as too tedious, and carried evaporation only to the point where alcohol was all expelled, acidifying the aqueous residue with normal acid and filtering as usual. He also washed the acid solution with ether before making alkaline and shaking out. A number of workers note the usual difficulty in filtering the acidified residue from evaporation. Mr. Hankey added powdered pumice to the residue to aid filtration and titrated finally with half-strength lime water. He found the marc on repeating the extraction yielded no more alkaloid. Mr. La Wall in a parallel experiment shook out finally with chloroform-ether mixture instead of ether and obtained lower results, viz, gravimetric 0.35 per cent and volumetric 0.416 per cent. Doctor Lyons used paper pulp to aid filtration, and after the final shaking out with ether further shaking out with chloroform yielded about 0.1 per cent alkaloid, titrating 0.075 per cent as aconitin and producing its characteristic effect on the tongue. He believes that aconite assays should be confirmed by the Squibb physiological test. He also suggests a direct titration method for aconite, similar to the

United States Pharmacopœia method for belladonna in the details of extracting the drug, but instead of shaking out the ethereal extract with acid, the former is to be evaporated, ammonia expelled by repeated addition of a few cubic centimeters of ether and evaporation, and the impure residue titrated. It might either be dissolved in alcohol diluted with water and titrated with acid, or dissolved in excess of standard acid and the excess of acid titrated with standard alkali, preferably with iodeosin indicator. This method, he thinks, could be adapted for many alkaloidal drugs. Professor Ruddiman criticises the use of decinormal acid, especially in titrating an alkaloid of such high molecular weight as aconitin, where a slight difference in measurement seriously affects the result.

In Method II as well as in I, Mr. Lyons obtained a further yield of about 0.1 per cent of alkaloid by shaking out with chloroform following the final extraction of the alkaline liquid with ether. Mr. Pearson redissolved the alkaloidal residues from the gravimetric determinations in (II) in acid and purified by submitting them to a shaking-out process with ether, obtaining much lower results, viz, 0.312 and 0.315 per cent.

In view of the fact that both methods gave practically the same average volumetric result and variability, the greater convenience and rapidity of Method II are in its favor.

#### BELLADONNA LEAVES.

This sample was delivered as No. 60 powder. The following proportions passed through the respective sieves:

	Grams.
No. 60.....	40
No. 50.....	35
No. 40.....	25
Total.....	100

A considerable amount of coarser powder than the Pharmacopœia permits in assay samples of belladonna leaves was present. By Method I (U.S.P.) the few gravimetric results reported varied exceedingly, none of them coming within 10 per cent of the average, and only 14 per cent within 15 per cent of the average. Of the volumetric results, 41 per cent came within 10 per cent and 65 per cent within 15 per cent of the average. By (II) gravimetric, 86 per cent of the few results were within 10 per cent; also 86 per cent within 15 per cent of the average. Of the volumetric results by (II) 39 per cent came within 10 per cent, and 73 per cent within 15 per cent of the average. The average results by (I) are slightly higher than by (II), but both are somewhat under the United States Pharmacopœia standard of 0.30 per cent. A slight impurity in the residues is indicated by the higher gravimetric results. In (I) Mr. Hankey used 2 cc of ether to assist solution of the alkaloidal residue in acid, expelling it by gentle warming before titration. J. G. Francis and Parker used 50 cc more ether-chloroform mixture than directed to exhaust the drug. It has been observed in the Drug Division when assaying belladonna leaves and root and coca leaves by the pharmacopœial method<sup>a</sup> that a large portion of the last 50 cc of solvent mixture which is intended to complete the percolation has to be used in rinsing the drug into the percolator. The drug should be packed after it is all transferred and percolation carried to practical exhaustion. The combined acid solutions obtained by shaking with the percolate should be shaken with fresh solvent in small portions until no more color is removed before making alkaline and shaking out the alkaloid. Instead of measuring out 3 cc of decinormal sulphuric acid to dissolve the alkaloid, a number of workers in such

<sup>a</sup> Workers in the Division of Drugs recommend cylindrical nursing bottles (8 ounces) which taper to the neck without any shoulder instead of Erlenmeyer flasks for digesting the drug with solvent, as the former are more easily clamped on a mechanical shaker.

cases prefer to add an equivalent amount of fiftieth-normal acid as a quantity less liable to error in measurement.

In (II) Mr. Blome suggests increasing the amount of ether-chloroform mixture for extracting the drug to 180 cc and decanting 120 cc. Mr. Fuller suggests that instead of directing the use of neutral alcohol for dissolving the alkaloid before titration it would be preferable to compare the result with that of a blank titration made with the same amount of the same stock of alcohol, water, and indicator. Mr. Hankey reports dissatisfaction with the titration results owing to an indefinite end reaction. Though his alcohol was redistilled over alkali, a blank titration with the amounts of acid, alcohol, and water directed required only 14.3 cc of fiftieth-normal alkali, while the same amount of acid by direct titration required 15 cc of the standard alkali. Mr. Parker prepared "neutral" alcohol by adding fiftieth-normal potassium hydroxid to alcohol until a blank titration with the amounts of acid, alcohol, and water directed agreed with a direct titration of the acid alone. This method or that suggested by Mr. Fuller eliminates the effect of any deviation from neutrality by the alcohol or water under the working conditions. Mr. Lyons made a parallel experiment, evaporating the ether-chloroform extract of the drug instead of shaking out with acid and titrating the residue directly, as outlined in the discussion under aconite root. The result was 0.32 per cent.

#### BELLADONNA ROOT.

This sample was delivered as No. 60 powder, and passed through the several sieves in the following proportions:

	Grams.
No. 80.....	98
No. 60.....	1
Total.....	99

It was, therefore, somewhat finer than the Pharmacopœia requires for assay samples of this drug. By Method I (U. S. P.) of the few gravimetric results 29 per cent came within 10 per cent of the average and 43 per cent within 15 per cent. Of the volumetric results, 46 per cent came within 10 per cent of the average and 80 per cent within 15 per cent. By (II) the gravimetric results varied more than the similar determinations by (I). The volumetric results by (II) were decidedly better than the corresponding results by (I), 73 per cent coming within 10 per cent of the average and 85 per cent within 15 per cent. The averages by the two volumetric determinations are practically identical, likewise those by the two gravimetric determinations, but no explanation is apparent for the fact that by both methods the gravimetric results average lower than the volumetric. This relation occurs also in four instances (in II) where the results apparently represent the same residue.

In (I) Mr. Hankey dissolved the alkaloidal residue in 1 cc of neutral alcohol before adding excess of standard acid and titrating back with half-strength limewater, comparing the same with a blank titration. C. H. La Wall made parallel assays by both methods, evaporating the ether-chloroform extract instead of shaking out with acid, and titrating the impure residue directly, the results obtained being (I) 0.514 and (II) 0.529 per cent, duplicate results agreeing well. J. G. Francis used 25 cc, and Mr. Parker 50 cc more ether-chloroform mixture than the amount directed to extract the drug. Their results are all well above the average. The remarks made in the discussion on belladonna leaves, Method I, regarding the percolation of the drug also apply to belladonna root. With belladonna root Method II, by evaporation of the ether-chloroform extract, and direct titration of the impure residue, Mr. Lyons obtained a value of 0.617 per cent.





## COCA LEAVES.

This sample, delivered as No. 60 powder, passed through the several sieves in the following proportions:

	Grams.
No. 60.....	29
No. 50.....	18
No. 40.....	9
No. 20.....	43
Total.....	99

A large portion of the powder was coarser than the pharmacopœial requirement for assay samples of this drug. The gravimetric results by Method I (U. S. P.) are too few to justify any conclusions. Of the volumetric results, 36 per cent come within 10 per cent and 58 per cent within 15 per cent of the average. Of the gravimetric results by (II), 75 per cent come within 10 per cent and 82 per cent within 15 per cent of the average. Of the volumetric results, 33 per cent come within 10 per cent and 72 per cent within 15 per cent of the average. The gravimetric averages and likewise the volumetric averages by the respective methods are in substantial agreement, the gravimetric results being somewhat higher than the volumetric, owing probably to impurities in the alkaloidal residue. In (I) Mr. Fuller accomplished the final shaking out with three portions of 20 cc each of ether instead of 25, 20, and 15 cc. He thinks the drug should be digested with the solvent mixture longer than one hour, as the marc in this case still contained alkaloid. Mr. Hankey dissolved the alkaloidal residue with 1 cc of "neutral spirits" and titrated with acid and diluted limewater as with belladonna root. Cochineal gave an unsatisfactory end reaction. Messrs. La Wall and Parker noted considerable emulsification in shaking out by both methods. The latter used 50 cc more solvent than is directed for percolating the drug, and J. G. Francis used 75 cc more, and shook the drug finally with five portions of 25 cc of ether. The extraction was not complete.

For coca as for belladonna the amount of solvent mixture directed in the United States Pharmacopœia method is scarcely adequate for the proper manipulation and extraction of the drug. In the final shaking out process further extraction with ether is desirable. In (II) Mr. Blome suggests increasing the ether-chloroform mixture to 180 cc and decanting 120 cc. Mr. Hankey obtained a better end reaction with iodeosin than with cochineal. Professor La Wall obtained equally low results in a duplicate assay. J. G. Francis found that the final extraction was not complete. Mr. Pearson could not decant 100 cc without forcible expression, and therefore objects to the method. As in (I), further extraction with ether in the final shaking out is probably desirable.

In both (I) and (II) considerable impurity evidently passes into the alkaloidal residue, and a more thorough washing with solvent before making alkaline is indicated.

## THE MACROSCOPY AND MICROSCOPY OF DRUGS.

By H. H. RUSBY.

The object of this brief paper is to direct the attention of the members to the importance of chemists supplementing their chemical methods by suitable physical methods in identifying and estimating drugs; and to the facility with which the chemist can acquire enough knowledge of such physical methods, and of the physical properties of drugs, to be of great assistance in his analytical work.

When the subject of the chemical standardization of vegetable drugs was being agitated in connection with the approaching United States Pharmacopœia Convention of 1890, the writer was astonished to hear Prof. John M. Maisch declare himself

opposed to the introduction of such standards into the Pharmacopœia. This surprise was considerably augmented when Doctor Maisch gave as his reason the statement that if a man knew drugs as he should it would not be necessary to examine them chemically to determine their quality. Although we can not in these days admit the propriety of neglecting chemical standardization, for this or any other reason, yet subsequent experience has shown that Doctor Maisch's claim to be able to judge the quality of drugs without recourse to chemical methods is largely justified.

The necessity of such knowledge is apparent when we reflect that of the 167 crude vegetable drugs of the Pharmacopœia, chemical standards are prescribed for only 22, and yet the Pharmacopœia does not recognize more than one-half of the nonstandardized articles in common use. It is true that chemists employ quantitative methods, all more or less satisfactory, in the case of ten or a dozen others, which are not thus treated in the Pharmacopœia. Admitting these to full membership, how overwhelming still is the majority upon the other side! Let it not be said that the non-assayable list represents only unimportant drugs. It is one of the great temptations of the chemist to underrate subjects with which he does not deal, and he is apt to reason *post hoc, ergo propter hoc*. Let us not forget that it is the extreme variability in activity of such drugs as *veratrum*, *digitalis*, *ergot*, and *cannabis indica*, coupled with their exceeding importance in medicine, which has forced a resort to physiological standardization, applicable as yet to but few drugs. It is this tendency to vary in quality and our general inability to estimate such quality that has to a great extent destroyed the usefulness of some drugs which would otherwise be generally relied upon. As illustrations, let us note male fern, *spigelia*, *cusso*, and other anthelmintics, *Winter's bark*, *coto bark*, and *chrysarobin*. The importance of the drugs named is relatively greater than that of the assayable ones, by virtue of the fact that the latter can be substituted by their proximate principles, while the former can not.

There is yet another element of weakness in the chemical assay of drugs, which is greatly mitigated by attention to their macroscopical and microscopical characters. Every assayer is frequently more or less chagrined by the thought that after all he does not know what it is that he has in hand after he has extracted the full required percentage of alkaloid by the prescribed method, since part of it may have been extracted from an admixture. Impurities in drugs, either from accident or design, may and frequently do fail of detection by the chemist, even in the case of freely assayable drugs, where detection would be simple by intelligent physical examination before assaying.

Even the great array of unofficial and unimportant drugs can not be dismissed from the chemist's ken because of their want of substantial therapeutic activity. They are in common use and some one pays for them the money which is his property and which entitles him to the receipt of what he pays for. He may be deprived of the protective aid of the Pharmacopœia without having his legal or professional rights in any degree curtailed. Indeed, the chemist himself is a deeply interested party in this class of transactions. Every commercial chemist will admit that some of his most profitable work lies in the field of the unofficial *materia medica*, and where the distinctly chemical indications are usually indefinite and faint. It seems quite unnecessary to argue further that a knowledge of the physical identification characters of vegetable drugs is of great service to the chemist. Is it too much to say that the field of success thus opened to him is far greater, as to crude vegetable drugs, than that which he can control by chemical methods alone? I feel very sure that such a statement is just and moderate.

This being so, how far can macroscopical and microscopical methods supply the deficiency? And how great an expenditure of effort and time does it require? It may be admitted at once that to secure an expert knowledge of this subject requires the same kind and degree of application that it does to become an expert chemist, but it is at the same time true that a very moderate amount of effort, intelligently

and judiciously applied, will add more to the general efficiency of the chemist than the same amount applied in any other direction. I believe that no chemist should proceed with the chemical examination of a drug of this class until after he has examined it physically, with or without the microscope, according to the requirements of the case, to ascertain its general characters and particularly whether it is a single article or a mixture. This requires a fair knowledge of macroscopy and microscopy, as to both methods and drugs. The time and labor necessary to acquire such a knowledge are not excessive. As to all the official and important unofficial drugs, it should be gained by from one hundred to one hundred and fifty hours of practical work, say two or three hours per week during a two-year course.

The following examples will serve to illustrate the class of drugs to which reference is here made: Coto and paracoto bark are among the most reliable therapeutic agents in the materia medica, often the only means of saving life in severe cases of dysentery, yet the use of this medicine has almost ceased owing to the fact that the genuine drug is now scarcely ever seen. In two years the writer has not known of an importation of it to the United States that was not spurious. A brief macroscopic examination will enable anyone immediately to recognize every one of these pretenders. The same statement applies, in a somewhat less serious degree, to Winter's bark, a most valuable aid in nutrition.

The belladonna invoice covers a multitude of fatal and dangerous imperfections. A very large part of our belladonna root contains poke root, not only an exceedingly active poison but an article that counteracts the medicinal effect of belladonna. It is sometimes difficult to distinguish the smaller roots by macroscopical means, but the dust in the package will always show, under the microscope, the needle-shaped crystals of the poke root. The same statement applies to an admixture of poke leaves to belladonna leaves. Scopola leaves are often mixed with and substituted for belladonna leaves. This is liable to destroy the life of the patient receiving the medicine. In any case the medicinal actions of these two are antagonistic. Some indication of the identity of these plants is almost always present with the leaves; for example, the belladonna has black berries, while the scopola has pale yellow circumscissile pods, and the two can be instantly distinguished.

A spurious henbane sometimes contains from ten to fifteen times as much alkaloidal matter as the genuine and has a different action. These alkaloids are so poisonous that they are given in doses of only one two-hundred-and-fiftieth to one one-hundredth of a grain. Imagine the effect of giving a dose containing fifteen times as much as it should. When powdered, the spurious can be recognized by its stellate hairs and by certain cells with wavy thick walls. Henbane and digitalis may contain stramonium leaves. Any considerable amount of such an addition to digitalis must put the life of the patient in danger, because with heart failure life often depends upon the full and prompt action of the latter remedy. Here the microscope is almost necessary, as a single hair from the leaf of the stramonium, densely covered with minute warts, will tell the story.

Strophanthus seed is another drug of great service in heart failure, and used when promptness is necessary. There is one variety of the seed which produces no good effect, and there has been ten times as much of this used in the United States as of the other, because it has cost only one-tenth to one-fifth as much. During the past year the use of the spurious kind has been largely stopped. The two seeds have such different macroscopic characteristics that they can not be mistaken when once the difference has been noted.

So-called saffron is frequently found which consists of marigold flowers, colored red with anilin and heavily weighted with mineral matter. The evil result of this fraud is peculiar. Saffron is largely used for giving an agreeable color to medicinal preparation, so it is added to medicines in a prescription. This mineral matter is



apt to destroy the effect of other substances in the mixture, and may easily bring about changes in them that will result in poisoning the patient.

Let us now turn to the distinctively microscopical class of examinations and observe the facility of identification. Starch grains taken from different drugs, under the microscope are as conspicuously different as are larger objects. The same is true when they are modified in appearance by moist heat. The presence of such grains often shows that the drug has been partly exhausted of its activity. Powdered elecampane illustrates a very large class of drugs that do not contain any starch. If we find starch grains in any of these powders, we know that there must be an admixture. The various forms of crystals of calcium oxalate are very distinctive, the particular form being always the same in a given drug. Merely glancing at the powder under the microscope would identify a drug by this means. Ground olive pits have been used to the extent of hundreds of tons for adulterating such important drugs as ipecac, gentian, belladonna, and aconite. While stone cells occur in many drugs, similar to those of the olive pit, they are absent from most, and their characteristic appearance is sufficient for ready detection. The very similar stone cells from coconut shells have been largely used to adulterate chocolate, but when compared with the powders of chocolate under the microscope they could not fail of detection.

Plant hairs are often so characteristic as to insure instant recognition. The stellate hairs of the chestnut leaf, one of the favorite articles used to adulterate medicinal leaves and herbs, are very distinctive; the peculiar hairs of stramonium and spurious henbane have already been mentioned. Genuine and spurious matico are easily distinguished, the latter having only about one-third the medicinal activity of the former. Its hairs are large, strong, and thick-walled, the cavity being little more than a faint line. The hair of the genuine, on the other hand, is nearly all cavity, its wall so thin that the hair frequently collapses.

It is earnestly hoped that this presentation of the subject may lead some here to interest themselves, at least a little, in this matter. The attention of this association has been chiefly directed to other things than drugs. Important as those subjects are, your aid is equally needed in the drug field. There are only a few of us to struggle with this great subject. Efforts to secure just action by the final authorities are met by the most energetic and often very plausible misrepresentations by interested parties, to the great detriment of the cause, and there is great need of your moral support in promoting public interest in the rigid enforcement of the laws regarding pure drugs.

## THIRD DAY.

### SATURDAY—MORNING SESSION.

Mr. J. P. Street introduced a resolution approving national legislation regulating the composition and sale of insecticides and fungicides. The matter was referred to the committee on resolutions. (See page 189.)

#### REPORT ON PHOSPHORIC ACID.

By J. M. McCANDLESS, *Referee.*

On May 19, 1908, the referee sent out a letter to twenty-one chemists, quoting the recommendations made by the association, as follows:

(1) That the referee on phosphoric acid take up for report at the next meeting of the association methods applicable under American conditions to the official examination of basic slag phosphates.

(2) That the subject of an accurate determination of iron oxid and alumina in rock phosphates be examined by the referee on phosphoric acid and an official method be recommended to the association next year.

(3) That a number of chemists be requested to send to the referee on phosphoric acid samples of the citrate ammonia solution employed by them, and that the referee examine such samples as to neutrality and that such examination be reported to the chemists at the next annual meeting.

In compliance with these instructions the referee requested those who desired to cooperate in the work to send him a bottle (200 cc) of their solution of ammonium citrate and a short statement of the method used in making the samples neutral.

In response to this letter the referee received nine samples of ammonium citrate solution for examination and forwarded to ten chemists three samples each, one of pulverized brown Tennessee rock, one of pulverized Florida rock, and one of a synthetic solution made from microcosmic salt, recrystallized potash-alum, ferrous ammonium sulphate, calcium carbonate, magnesium sulphate, and calcium fluorid, so that 100 cc would represent 1 gram of substance, and on that basis the solution should contain exactly 3 per cent of ferric oxid and 2 per cent of alumina.

A letter of instructions was forwarded with the samples requesting that the cooperators test the following methods for iron and alumina, it being deemed best to restrict the work to these phases:

#### METHODS FOR THE DETERMINATION OF IRON AND ALUMINA IN PHOSPHATE ROCK.

It is recommended that before beginning the work each analyst make up for himself a synthetic solution from C. P. chemicals, containing 10 grams of microcosmic salt, 10.4 grams of calcium carbonate, 0.050 gram of magnesium oxid or its equivalent in magnesium sulphate, 0.300 gram calcium fluorid. To these should be added accurately known weights of C. P. crystallized potash, or ammonia alum, and ferrous ammonium sulphate or iron wire. The material should be dissolved in hydrochloric acid and water and made up to a liter. The methods should be tried upon this solution to acquire confidence and applied to the referee's samples, using the following methods:

*Gladding method.*

Dissolve 4 grams of the rock in 30 cc dilute hydrochloric acid (1 to 1), heating just below the boiling point for half an hour. Filter into a 200 cc flask, add a few drops of nitric acid, and boil to oxidize the iron; cool and dilute to mark. Take 50 cc, containing 1 gram, and run into 20 cc of a solution of C. P. caustic potash, made by dissolving 500 grams of caustic potash free from alumina, in distilled water and diluting to one liter. Digest in water bath at 70° for one hour, stirring occasionally. Let the precipitate settle and filter on a large paper, first decanting the supernatant liquid on the paper and finally washing on the precipitate. Wash two or three times with hot water. To the filtrate add 1 gram of ammonium phosphate; acidify with hydrochloric acid. Add ammonia until a permanent precipitate is formed; add dilute hydrochloric acid, drop by drop, until it is just dissolved.

Add a mixture of 15 cc neutral ammonium acetate solution and 5 cc acetic acid (30 per cent) and digest for half an hour at 70° C., by which time the precipitation is complete. Filter, washing five or six times with hot ammonium acetate solution (10 per cent), stirring up the precipitate with the jet each time. Ignite with a low flame till the paper is charred, increase the heat until the paper is consumed, then blast for a minute.

The precipitate is  $AlPO_4$ , and its weight multiplied by 0.418 gives the  $Al_2O_3$ . Gladding determines the iron oxid volumetrically by the bichromate method in a solution of the precipitate of iron oxid and calcium phosphate thrown down by the caustic potash, or by the same method in a separate solution of 5 grams of the rock in dilute hydrochloric acid (1 to 1).

*Glaser method.*

Boil 3 grams of phosphate rock thirty minutes in 30 cc concentrated hydrochloric acid. Make up to 300 cc and filter off 100 cc. Add 25 cc concentrated sulphuric acid; shake and allow to stand a few minutes; add 100 cc strong alcohol and cool. Make up to 250 cc with alcohol and allow to stand thirty minutes; filter off 100 cc or 0.4 gram and evaporate in a large beaker to expel alcohol.

Transfer to a small Griffin beaker, boil, remove from flame, and make slightly alkaline with ammonia. Boil to neutrality, cool, filter, and wash with boiling ammonium nitrate solution. Burn and weigh, weight divided by 2 = oxids of iron and aluminum.

*Proposed modification of acetate method.*

Weigh 2.5 grams of phosphate rock into a 250 cc flask; cover with 25 cc of concentrated hydrochloric acid; keep just below the boiling point for thirty minutes; dilute and cool; make up to the mark; filter off 50 cc, equivalent to one-half gram of rock; add a few drops of nitric acid, to oxidize any ferrous iron, and boil.

Add ammonia until the precipitate formed dissolves slowly on agitation. Then cool to about 15° C., neutralize, adding dilute ammonia drop by drop until the precipitation is complete. Clear up with dilute hydrochloric acid added drop by drop, slowly and with frequent shaking toward the last until the solution is clear. Make a solution of ammonium acetate by neutralizing strong ammonia with acetic acid sp. gr. 1.04; to 15 cc of this solution add 5 cc of acetic acid, sp. gr. 1.04, in a tall beaker having a capacity of about one liter; fill the beaker about seven-eighths full with hot water, so that the mixture will have a temperature of 70° to 75° C.; pour the solution of phosphate in a thin stream into the dilute hot solution of the ammonium acetate, stirring constantly. The precipitated phosphates of iron and aluminum are allowed to settle, and after becoming clear the greater part of the supernatant fluid is siphoned off, the beaker is filled up again with hot water at about 70°, again allowed to settle, and the supernatant fluid is siphoned off.

The remainder in the beaker is now filtered off on a large, rapid filtering paper (S. & S. black band ashless) washed thoroughly with hot water containing ammonium nitrate, keeping the precipitate on the filter well stirred up with a strong jet from the wash bottle. Ignite at a low temperature, till the paper is charred, increase heat until the paper is fully consumed, and finally blast for a minute. The weight of the precipitate in centigrams gives the percentage of the mixed oxids.

It is desired that in the last two methods the percentage of the mixed oxids of iron and alumina be given and also that the oxid of iron be determined separately by any volumetric method preferred by the analyst, always observing the precaution of oxidizing the organic matter to be found in solutions of phosphate rock by digesting with potassium chlorate and boiling off the excess of chlorine previous to the reduction and titration.

The referee desires to remind the analysts cooperating in this work that it has been undertaken under the auspices of the A. O. A. C. for the purpose of establishing, if possible, a standard method for the estimation of iron and alumina which should have the indorsement of the association. At present all is chaos, and when two chemists differ on iron and alumina it is impossible to say who is right and who is wrong.

The great majority of rock sales to-day are settled either by Gladding method or by the Glaser method, as outlined above.

The referee submits the above modification of the acetate method, which he believes to be simpler and fully as accurate as the others, and will welcome the comments and criticisms of the analysts when they have completed the work on the three samples by the methods outlined above. In regard to the synthetic solution sent, the referee would say that it was not practicable to send more than 300 cc to each analyst, but that in his opinion one-half gram, or 50 cc, is sufficient for any of the tests required, and used in this way there is sufficient of the synthetic solution for six tests.

Reports were received from six chemists cooperating in the work on iron and alumina, whose results are given in the following tables:

*Determination of iron and alumina in Tennessee and Florida rock.*

TENNESSEE ROCK.

Analyst.	Determination.	Method of—				
		Gladding.	Glaser.	McCandless.	Veitch.	Von Grueber.
Stillwell and Gladding, New York City.	$Fe_2O_3$ .....	<i>Per cent.</i> 2.79-2.79	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
	$Al_2O_3$ .....	3.50-3.44				
	Total.....	6.29-6.23	6.80	6.20		
	Average.....	6.26		6.10		
F. B. Carpenter, by R. Henry, Richmond, Va.	$Fe_2O_3$ .....	3.17				
	$Al_2O_3$ .....	3.50				
	Total.....	6.67		6.48	7.20	
G. Farnham, Jarecki Chemical Co.	$Fe_2O_3$ .....	3.06	3.06			
	$Al_2O_3$ .....	2.29	3.92			
	Total.....	<i>a</i> 5.35	6.98			
P. Rudnick, by G. F. Beyer, Chicago, Ill. <sup>b</sup>	$Fe_2O_3$ .....	3.22				3.22
	$Al_2O_3$ .....	3.21				3.68
	Total.....	6.43	7.04	6.19		6.90
	Average.....					
McCandless, Burton, and Atkinson, Atlanta, Ga.	$Fe_2O_3$ .....	3.04				
	$Al_2O_3$ .....	3.26				
	Total.....	6.30	6.48	6.22		
S. H. Wilson, Georgia Department of Agriculture.	$Fe_2O_3$ .....	2.91				
	$Al_2O_3$ .....	3.21				
	Total.....	6.12	5.86	5.90		
O. M. Shedd, Kentucky station.	$Fe_2O_3$ .....		2.92-2.92			
	$Al_2O_3$ .....					
	Total.....		6.39-6.49	4.32-4.13		
Average.....		6.44	<i>a</i> 4.22			
Average.....		6.35	6.60	6.19		

<sup>a</sup> Omitted from average.

<sup>b</sup> Per cent calculated from average of three determinations.

## Determination of iron and alumina in Tennessee and Florida rock—Continued.

## FLORIDA ROCK.

Analyst.	Determination.	Method of—				
		Gladding.	Glaser.	McCandless.	Veitch.	Von Grueber.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Stillwell and Gladding, New York City.	Fe <sub>2</sub> O <sub>3</sub> .....	1.71	.....	1.70-1.75	.....	.....
	Al <sub>2</sub> O <sub>3</sub> .....	1.49	.....	.92-1.00	.....	.....
	Total.....	3.20	3.51	2.62-2.75	.....	.....
	Average.....	.....	.....	2.68	.....	.....
F. B. Carpenter, by R. Henry Richmond, Va.	Fe <sub>2</sub> O <sub>3</sub> .....	1.85	.....	.....	.....	.....
	Al <sub>2</sub> O <sub>3</sub> .....	.94	.....	.....	.....	.....
	Total.....	2.79	.....	2.73	2.89	.....
G. Farnham, Jarecki Chemical Co.	Fe <sub>2</sub> O <sub>3</sub> .....	1.65	1.65	.....	.....	.....
	Al <sub>2</sub> O <sub>3</sub> .....	.66	1.33	.....	.....	.....
	Total.....	2.31	2.98	.....	.....	.....
P. Rudnick, by G. F. Beyer, Chicago, Ill. <sup>a</sup>	Fe <sub>2</sub> O <sub>3</sub> .....	1.86	.....	.....	.....	1.86
	Al <sub>2</sub> O <sub>3</sub> .....	1.03	.....	.....	.....	1.53
	Total.....	2.89	3.48	1.95-1.97	.....	3.43
	Average.....	.....	.....	<sup>a</sup> 1.96	.....	.....
McCandless, Burton, and Atkinson, Atlanta, Ga.	Fe <sub>2</sub> O <sub>3</sub> .....	1.73	.....	.....	.....	.....
	Al <sub>2</sub> O <sub>3</sub> .....	.94	.....	.....	.....	.....
	Total.....	2.67	3.90	2.72	.....	.....
S. H. Wilson, Georgia Department of Agriculture.	Fe <sub>2</sub> O <sub>3</sub> .....	1.68	.....	.....	.....	.....
	Al <sub>2</sub> O <sub>3</sub> .....	.90	.....	.....	.....	.....
	Total.....	2.58	2.96	2.62	.....	.....
O. M. Shedd, Kentucky station.	Fe <sub>2</sub> O <sub>3</sub> .....	.....	1.68-1.66	.....	.....	.....
	Al <sub>2</sub> O <sub>3</sub> .....	.....	1.67	.....	.....	.....
	Total.....	.....	2.96-2.98	<sup>a</sup> 1.50-1.69	.....	.....
	Average.....	.....	2.97	.....	.....	.....
Average.....	.....	2.74	3.30	2.69	.....	.....

<sup>a</sup> Per cent calculated from average of three determinations.

## Determination of iron and alumina in synthetic solution.

[Synthetic solution—made to contain 3 per cent  $\text{Fe}_2\text{O}_3$  and 2 per cent  $\text{Al}_2\text{O}_3$ , or 5 per cent combined oxids.]

Analyst.	Determination.	Method of—				
		Gladding.	Glaser.	McCandless.	Veitch.	Von Grueber.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Stillwell and Gladding, New York City.	$\text{Fe}_2\text{O}_3$ .....	2.60	.....	2.50	.....	.....
	$\text{Al}_2\text{O}_3$ .....	3.68	.....	2.73	.....	.....
	Total.....	<sup>a</sup> 6.28	<sup>a</sup> 6.12	5.23	.....	.....
F. B. Carpenter, by R. Henry, Richmond, Va.	$\text{Fe}_2\text{O}_3$ .....	.....	.....	.....	.....	.....
	$\text{Al}_2\text{O}_3$ .....	.....	.....	.....	.....	.....
	Total.....	.....	.....	5.01	5.83	.....
G. Farnham, Jarecki Chemical Co.	$\text{Fe}_2\text{O}_3$ .....	3.08	3.08	.....	.....	.....
	$\text{Al}_2\text{O}_3$ .....	1.86	1.86	.....	.....	.....
	Total.....	4.94	4.94	.....	.....	.....
P. Rudnick, by G. F. Beyer, Chicago, Ill. <sup>b</sup>	$\text{Fe}_2\text{O}_3$ .....	3.11	.....	3.11	.....	.....
	$\text{Al}_2\text{O}_3$ .....	2.15	.....	1.50	.....	.....
	Total.....	5.26	5.62	4.61	.....	.....
McCandless, Burton, and Atkinson, Atlanta, Ga.	$\text{Fe}_2\text{O}_3$ .....	3.05	.....	.....	.....	.....
	$\text{Al}_2\text{O}_3$ .....	2.06	.....	.....	.....	.....
	Total.....	5.11	5.55-5.60 (5.57)	5.07-5.05 (5.06)	.....	.....
S. H. Wilson, Georgia Department of Agriculture.	$\text{Fe}_2\text{O}_3$ .....	2.91	.....	.....	.....	.....
	$\text{Al}_2\text{O}_3$ .....	2.55	.....	.....	.....	.....
	Total.....	5.46	5.40	5.18	.....	.....
O. M. Shedd, Kentucky station.	$\text{Fe}_2\text{O}_3$ .....	.....	3.12-3.12 (3.12)	.....	.....	.....
	$\text{Al}_2\text{O}_3$ .....	.....	.....	.....	.....	.....
	Total.....	.....	4.92-4.98 (4.95)	4.10-4.38 <sup>a</sup> (4.24)	.....	.....
Average.....	.....	5.28	5.29	5.02	.....	.....

<sup>a</sup> Omitted from average.<sup>b</sup> Per cent calculated.

The determination of iron and alumina by various modifications (Shedd),<sup>a</sup>

## GLADDING METHOD.

Modification.	Florida rock (25198).	Tennessee rock (25199).	Referee's synthetic solution (25200), 50 cc.	Shedd's synthetic solution, 50 cc.
$Al_2O_3$ by subtracting $FePO_4$ from weight of $FePO_4 + AlPO_4$ and multiplying by 0.419:				
First determination .....	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Second determination .....				0.0108
Mean .....				.0111
Theory .....				.0110
$Fe_2O_3$ obtained from the precipitate of $FePO_4 + AlPO_4$ obtained above:				
First determination .....				.0193
Second determination .....				.0195
Mean .....				.0194
$Fe_2O_3$ obtained from independent portions of 50 cc of the solution:				
First determination .....				.0194
Second determination .....				.0194
Mean .....				.0194
Theory .....				.0194

## MODIFIED ACETATE METHOD.

$Al_2O_3$ by subtracting $FePO_4$ , etc., as above:				
First determination .....	-0.10	1.25	0.0051	-0.0020
Second determination .....	-.05	1.09	.0058	
Mean .....	-.025	1.17	.0055	
$Fe_2O_3 + Al_2O_3$ by halving the weight of phosphates:				
First determination .....	1.50	4.32	.0205	.0158
Second determination .....	1.69	4.13	.0219	
Mean .....	1.60	4.23	.0212	
$Fe_2O_3$ from independent 50 cc portions of the solution:				
First determination .....	1.74	3.03	.0157	
Second determination .....	1.69	2.94	.0157	
Mean .....	1.72	2.99	.0157	

## GLASER METHOD.

$Al_2O_3$ by subtracting $FePO_4$ , as above:				
First determination .....	1.16	3.05	0.0082	0.0047
Second determination .....	1.17	3.13	.0084	
Mean .....	1.17	3.09	.0083	
$Fe_2O_3 + Al_2O_3$ by halving the weight of phosphates:				
First determination .....	2.96	6.39	.0246	.0239
Second determination .....	2.98	6.49	.0249	
Mean .....	2.97	6.44	.0248	
$Fe_2O_3$ from independent 50 cc portions of the solution:				
First determination .....	1.68	2.92		
Second determination .....	1.66	2.92		
Mean .....	1.67	2.92		

<sup>a</sup> Analyses made by O. M. Shedd, of the Kentucky station, but received too late to incorporate in the report.

## DISCUSSION OF RESULTS.

*P. Rudnick (results by G. F. Beyer):* Commenting on the results in general, I do not believe that any of the methods proposed are preferable to the modified Von Grueber method in point of simplicity, rapidity, accuracy, and general applicability to various kinds of rock. Although the results obtained for ferric oxid in the synthetic solution prepared in this laboratory are high, they agree very well with the results obtained by the determination of iron in the precipitate from the method proposed by you, and the results on aluminum by the modified Von Grueber method are certainly very close to the calculated quantity. Although I have not had time nor opportunity to prove the point, I am inclined to believe that ammonium acetate is not sufficient to prevent the partial hydrolysis of the aluminum phosphate, and that ammonium nitrate is more efficient in this respect. I believe the fairly good agreement between the results by the Glaser method and the modified Von Grueber method obtained in this work, as well as at other times, supports this view.

*S. H. Wilson:* For simplicity and ease of execution the McCandless laboratory method leaves little to be desired.

*Remarks by the referee:* On the whole the results seem to be encouraging and to show that all three of the methods for which instructions were sent are capable of giving good results. One analyst used the Von Grueber method, another the Veitch method. A study of the results on the synthetic solution, in which the percentages of iron and alumina are accurately known, reveals a tendency on the part of those getting the lowest results on the phosphate rocks to get them also on the solution and vice versa; excluding the lowest and highest results, the agreements and approximations to the truth are about as good as would be found in the determination of other elements, as, for instance, phosphoric acid by the accepted methods.

The referee would call attention to the fact that this subject has been taken up by the National Fertilizer Association, and would recommend cooperation between the next referee and the committee of that association, with a view to reaching a decision as to what method shall be adopted.

#### EFFECT OF DILUTE AND CONCENTRATED HYDROCHLORIC ACID ON PYRITES IN PHOSPHATE ROCK.

The referee also requested the analysts cooperating to test the effect of dilute (1 to 1), and concentrated hydrochloric acid as to its solvent effect on pyrites, present to a greater or less extent in nearly all phosphate rock.

It has been claimed on the one hand that dilute hydrochloric acid (1 to 1) fails to dissolve all the iron and alumina, especially when the latter is present in the form of clay; it has been claimed, on the other hand, that concentrated hydrochloric acid, while it dissolves the alumina and iron oxids better than the dilute, also decomposes pyrites present in the phosphate rock and therefore yields too high a percentage of iron. It is desired that the analysts test this latter point as follows:

Procure a sample of freshly pulverized pyrites and weigh half a gram into a 250 cc flask, cover with 25 cc of hydrochloric acid (1 to 1), heat just below boiling for thirty minutes, dilute with 100 cc of water, shake, allow to settle, decant the liquid, repeat the washing by two or more treatments with 125 cc of cold water slightly acidulated with hydrochloric acid, followed by decantation. This preliminary treatment is to remove any oxid or sulphate of iron already existing in the pyrites. Have ready 2.5 grams of phosphate rock, add it to the flask on top of the washed pyrites, then cover with 30 cc of concentrated hydrochloric acid, heat just below boiling for thirty minutes, cool and make up to the mark. Determine the iron volumetrically in an aliquot of the solution and compare the results with that obtained from a similar treatment of phosphate rock and pyrites with dilute hydrochloric acid (1 to 1) the second time.

Only two chemists beside the referee took part in this work.



## Solvent effect of dilute and concentrated hydrochloric acid on pyrites.

Analyst.	Phosphate rock.	2.5 grams rock and 2.5 grams pyrites.	
		Concentrated HCl.	Dilute HCl.
G. F. Beyer, Chicago, Ill. ....	3.51-3.51	3.5-3.60	3.60-3.51
G. Farnham, Jarecki Chemical Co. ....	.....	2.91	3.74
J. M. McCandless, Atlanta, Ga. ....	3.48-3.50	3.49-3.52	3.50-3.47

While one of the results in the above table must be explained, the referee is convinced from a number of tests made years since that neither dilute nor concentrated hydrochloric acid has any appreciable effect on pyrites, and would therefore recommend the use of the concentrated acid in the solution of phosphate rock, heating for a definite time.

Of course, the use of sulphuric acid of 50° B. (which also has no action on pyrites), followed by solution in dilute hydrochloric acid, would more nearly approximate actual conditions, and it might be well if the next referee would investigate this method of solution as compared with simple solution in concentrated hydrochloric acid.

## EXAMINATIONS OF SOLUTIONS OF AMMONIUM CITRATE FOR NEUTRALITY.

As the referee was to decide whether the solutions were neutral or not, and as no two chemists agree on the exact point of neutrality, whether from lack of sensitiveness of the indicators, or color-blindness on the part of the operators, he decided to make an analysis of each sample according to the method outlined in his last report to the association, and be guided by those results in deciding upon neutrality. The following method of analysis was adopted:

Twenty-five cubic centimeters of each solution was pipetted into a 250 cc flask, made to mark, shaken, and 25 cc pipetted into a distillation flask; to the solution in the flask, 40 cc of fourth-normal caustic soda solution were added, and the contents of the flask distilled into 20 cc of half-normal acid, continuing the distillation until the volume of the distillate measured from 65 to 70 cc. The ammonia in the distillate was then titrated by means of tenth-normal alkali. The residue in the retort was washed into an Erlenmeyer flask, excess of standard acid added, then a few drops of phenolphthalein, and the excess estimated by means of tenth-normal alkali. From the result the weight of citric acid originally combined with the ammonia was calculated. Calculating from the formula of the pure salt,  $(\text{NH}_4)_3\text{C}_6\text{H}_5\text{O}_7$ , that the ratio of ammonia ( $\text{NH}_3$ ) to citric acid was as 1 to 3.765, a basis of comparison was established. The results obtained are given in the table below. As only three official chemists sent their solutions, the analysts are designated by number and not by name.

## Determination of neutrality of ammonium citrate solutions.

Number of analyst.	Ammonia in 25 cc of diluted solution=2½ cc original.	Citric acid in 25 cc of diluted solution=2½ cc original.	Ratio of ammonia to citric acid.	Ratio in neutral salt $(\text{NH}_4)_3\text{C}_6\text{H}_5\text{O}_7$ .	Reaction with corallin.
	<i>Milligrams.</i>	<i>Milligrams.</i>			
1.....	113.9	433.2	1:3.803	1:3.765	Neutral.
2.....	109.3	412.8	1:3.775	1:3.765	Alkaline.
3.....	104.9	424.96	1:4.051	1:3.765	Acid.
4.....	113.7	433.9	1:3.816	1:3.765	Neutral.
5.....	110.5	430.08	1:3.891	1:3.765	Slightly acid.
6.....	111.5	436.48	1:3.915	1:3.765	Acid.
7.....	108.7	421.1	1:3.874	1:3.765	Slightly acid.
8.....	104.7	398.7	1:3.808	1:3.765	Neutral.
9.....	102.8	430.7	1:4.189	1:3.765	Acid.

In this table all the solutions which showed materially more citric acid than 3.765 parts to 1 of ammonia also showed a decidedly acid reaction to corallin.

It appears that some chemists prepare their ammonium citrate solution by treating the citric acid with excess of ammonia and then leave the hot solution to neutralize itself, or finally adjust it, some by means of red and blue litmus paper, others by corallin. Some state that they have never been successful in the use of corallin; others adjust finally by means of the alcoholic solution of calcium chlorid. In the opinion of the referee, if a chemist has succeeded in getting his solution neutral or practically so, he will almost certainly put it out of joint by attempting to make it exact with the calcium chlorid solution. The referee finds that an alcoholic calcium chlorid solution which is exactly neutral to corallin is acid to phenolphthalein, and alkaline to cochineal; that after the precipitation of the citric acid from 10 cc of the ammonium citrate solution by 50 cc of the calcium chlorid solution, calcium citrate still remains in solution in the filtrate, which may be proved by boiling some of the clear solution, when a precipitate of calcium citrate will appear. The presence of this salt, in the opinion of the referee, renders the use of cochineal as indicator unreliable. One of the solutions in the above table, which is the most acid of all by analysis, was neutralized in this way. There are materials (notably fertilizers containing bone) on which a slight difference in neutrality of the ammonium citrate solution makes a great difference in the results. It is a reproach to the association that it has suffered the matter to remain in its present condition so long. While the referee has a strong personal conviction that the only proper method of making the solution neutral is by analysis and calculation of the exact quantity of ammonia or citric acid to be added to it, still he hesitates to urge it officially, as no work has yet been done by any other referee along this line, and because the referee is himself no longer an official chemist.

The referee desires to acknowledge the valuable aid and suggestions of Mr. J. Q. Burton in all of this work and the analytical assistance of Mr. F. C. Atkinson.

### THOMAS SLAG.

By J. B. LINDSEY.

Thomas slag or basic phosphatic slag is a by-product in the modern method of steel manufacture from ores containing noticeable quantities of phosphorus. The process of removing the phosphorus from the ore was discovered by the English engineers Gilchrist and Thomas and, briefly stated, consists in adding to the so-called "converter" containing the molten ore a definite quantity of freshly burned lime, which after a powerful reaction is found to be united with the phosphorus and swims upon the surface of the molten steel in the form of a slag.

#### COMPOSITION.

The composition of the Thomas or Belgian slag varies according to the character of the ore and the success of the process for removing the impurities. The following figures show such variations: <sup>a</sup>

	Per cent.
Phosphoric acid.....	11-23
Silicic acid.....	3-13
Calcium oxid (lime).....	38-59
Ferrous and ferric oxids.....	6-25
Protoxid of manganese.....	1- 6
Alumina.....	0. 2- 3. 7
Magnesia.....	2- 8
Sulphur.....	0. 2- 1. 4

<sup>a</sup> Adolf Mayer, *Agricultur Chemie*, 6th ed., vol. 2, pt. 2, pp. 138-139.

More or less metallic iron is inclosed in the coarse slag which is generally thoroughly removed from the ground material by the magnet.

#### MANURIAL VALUE.

The manurial value of the slag was not recognized for a long time. Finally experiments revealed that a considerable portion of its phosphoric acid was soluble in dilute citric and carbonic acids, which led to successful field experiments. The only preparation of the slag for fertilizing purposes, when its value was first recognized, consisted in having it finely ground in especially prepared mills, so that 75 per cent would pass through a sieve with perforations 0.17 mm in diameter. This requirement was suggested by M. Fleischer, who used the slag with much success in improving the condition of marsh and meadow lands.

#### METHODS FOR DETERMINING AVAILABILITY AND ADULTERATION.

Previous to 1890, by means of pot experiments, as well as by laboratory investigations, Wagner demonstrated that the phosphoric acid in different slags of the same degree of fineness varied in its availability from 30 to 90 or more per cent, and, further, that many brands were adulterated with Belgian or other insoluble mineral phosphates. The method therefore of determining the value of a slag by the percentage of total phosphoric acid present and the degree of fineness was of secondary importance.

In order to detect adulteration with mineral phosphates, Wagner originally used a dilute solution of citrate of ammonia and free citric acid.<sup>a</sup> The phosphoric acid in all of the mineral phosphates was sparingly soluble in such a reagent, while an unadulterated, high-grade slag gave up 80 to 90 parts of its phosphoric acid. Further investigations on various soils with many brands of slag made clear that the results obtained from pot experiments corresponded quite well with those secured by means of the citric-acid solution. This may be illustrated as follows:

#### *Determination of the availability of phosphoric acid.*

Brand of slag.	Phosphoric acid available.	
	In citric-acid solution.	In pot experiments.
1	100	100
2	85	80
3	81	72
4	72	72
5	73	66
6	76	63
7	39	40
8	48	38
9	42	38
10	45	31
11	38	30

Results similar to these were secured by Maereker,<sup>b</sup> who stated that "the results removed all doubt that the citrate solubility and plant experiments were so nearly proportioned that one had the same right to value the slag according to its content of phosphoric acid soluble in citrate solution as to value the superphosphate by its content of water-soluble phosphoric acid."

<sup>a</sup> Chemiker Zeitung, 1895, No. 63; also Düngungsfragen, 1896, No. 1, p. 16.

<sup>b</sup> Landw. Presse, 1895, No. 82.

As a result of these investigations the union of German experiment stations at its meeting at Kiel, in September, 1896, adopted the method <sup>a</sup> of determining the relative value of the slag according to its phosphoric acid solubility in a 2 per cent citric acid solution and did away with the previous standard of total phosphoric acid and fineness.

Wagner, as well as Maercker, repeatedly called attention to the fact that experiments both in the laboratory and with plants gave positive evidence that those slags of like phosphoric acid content which were richest in silicic acid gave the best results. G. Hoyermann, working independently, came to similar conclusions. At the present time, according to Wagner, practically all of the iron works treat the molten slag as it flows from the converter with hot quartz sand, with the result that the availability of the phosphoric acid is increased from 10 to 30 per cent.<sup>b</sup>

#### CHEMICAL COMBINATION OF PHOSPHORIC ACID IN SLAG.

The form in which the phosphoric acid exists in the slag has never been fully explained. It was formerly supposed that it was combined with lime as a tetracalcium phosphate and the latter being less stable than tricalcium phosphate became easily available to the plants by being decomposed, under the influence of dilute acids, into the calcium salt of the dissolving acid and dicalcium phosphate. The tetralime phosphate, however, has never been made artificially,<sup>c</sup> although it has been recognized by the aid of the microscope in the slag and exists as a mineral under the name of isoklas.

More recent investigations having shown, as already indicated, that those slags richest in silicic acid of like phosphoric acid content gave the best results, the conclusion followed that a part of the lime must be in the form of lime silicate. It is now generally held, especially by Wagner,<sup>d</sup> that the phosphoric acid is combined in the slag as a double salt of tricalcium phosphate and calcium silicate and that in this form the roots are able to utilize it. It is also believed probable that some of the phosphoric acid is more or less united with iron as a basic iron phosphate.

#### THE USE OF PHOSPHATIC SLAG.

Basic slag has been shown to work especially well upon sour marsh and meadow lands, upon porous, well-aired soils rich in humus, and upon sandy soils deficient in lime.

When a rapid development of the crop is not desired, the slag may be used exclusively in place of acid phosphate. On the other hand, in cases when it is feared that the crop will not mature early enough upon heavy, cold land and in high altitudes where the season is short, acid phosphate should be given the preference.

The phosphoric acid in slag is comparable in its quickness of action to nitrogen in barnyard manure, tankage, and green crops; and the phosphoric acid in acid phosphate to the action of nitrogen in nitrate of soda. A combination of slag and sulphate of potash (500 pounds of slag and 100 pounds of potash) has been found to work especially well upon grass land and to be very favorable to the development of clover.

<sup>a</sup> Method slightly modified from the original. Present method described in König's *Untersuchung landwirtschaftlich und gewerblich wichtiger Stoffe*, 3d ed., pp. 173-174.

<sup>b</sup> *Loc. cit.*; also Wagner, *Anwendung künstlicher Düngemittel*, pp. 74-75.

<sup>c</sup> Hilgenstock, *Jahresber. chem. Technologie*, 1887, p. 282, after Adolf Mayer, *loc. cit.*

<sup>d</sup> *Loc. cit.*

## QUANTITY OF SLAG PER ACRE.

If the soil is particularly deficient in phosphoric acid, one can use as high as from 800 to 900 pounds of slag to the acre, plowed in and supplemented with 200 pounds of acid phosphate in the hill or drill.

If, on the contrary, the soil is naturally rich in phosphoric acid or has been made so by large additions of slag for several consecutive years (1,000 or more pounds yearly) then it is necessary only to replace from year to year the amount removed by the crop. In such cases Maercker states that one part of phosphoric acid in basic slag is as valuable as an equal amount in acid phosphate.

## VALUATION OF PHOSPHORIC ACID IN BASIC SLAG.

By H. D. HASKINS.

Thomas basic slag is being used in Massachusetts and some of the other New England States more extensively each year, and it is therefore highly desirable that more satisfactory methods of analyzing and valuing this product be worked out by the association.

At the last meeting of the association the referee on phosphoric acid recommended that the phosphoric acid in Thomas basic slag be valued by the degree of fineness of the slag along the same lines as are employed in the case of ground bone, but the recommendation did not specify the diameter of the openings in the sieve used in the mechanical separation. If valued by its fineness the diameter of the openings in the sieve used for this purpose is obviously of great importance, as is shown by the following results: Two samples of slag were analyzed mechanically by the use of 100-, 50-, and 25-mesh sieves, the latter having circular openings one-fiftieth of an inch in diameter.

*Comparison of amount and value of phosphoric acid available by using sieves of different sized mesh.*

Sam- ple.	Total P <sub>2</sub> O <sub>5</sub> .	Mechanical analysis.						Valuation.		
		100-mesh sieve.		50-mesh sieve.		25-mesh sieve.		100-mesh sieve.	50-mesh sieve.	25-mesh sieve.
		Fine.	Coarse.	Fine.	Coarse.	Fine.	Coarse.			
	<i>Per cent.</i>	<i>Per ct.</i>	<i>Per cent.</i>	<i>Per ct.</i>	<i>Per cent.</i>	<i>Per ct.</i>	<i>Per cent.</i>	<i>Dollars.</i>	<i>Dollars.</i>	<i>Dollars.</i>
1	17.45	68.47	31.53	89.64	10.36	93.72	6.28	12.86	13.59	13.74
2	17.96	71.83	28.17	90.74	9.26	94.97	5.03	13.36	14.04	14.18

An average of the two samples shows that 94.34 per cent of the slag passes a 25-mesh sieve, 90.19 per cent passes a 50-mesh sieve, and only 70.15 per cent passes a 100-mesh sieve. Looking at the matter from the point of valuation, we find that allowing 4 cents for the phosphoric acid in the fine and 3 cents for the phosphoric acid in the coarse slag, the average valuation of the two samples of slag by use of the 25-mesh sieve would be \$13.96, by use of the 50-mesh sieve \$13.81, and by use of the 100-mesh sieve only \$13.11; a difference of nearly \$1 per ton in the extremes.

The next question to be considered is whether this method of valuing the phosphoric acid in basic slag is a safe one for us to adopt. During the month of August the writer's attention was called to a product put forth in large quantities in northern New York. The material was a waste product said to be largely apatite and quartz, coming from iron ore used in that locality. It was finely ground, 69.44 per cent passing a 100-mesh sieve, and contained 16.78 per cent of total phosphoric acid; the phos-

phoric acid in this material, however, by the Wagner method of analysis showed only 1.029 per cent of available phosphoric acid or 6.13 per cent of the whole, while the basic slag by this same method showed 15.48 per cent of available phosphoric acid or 87.4 per cent of the whole. The point in making mention of this apatite is to show that in case the phosphatic slag is adulterated with material of this nature the mechanical method of valuing the slag would prove decidedly misleading, and it is because of this that the method of valuing the phosphoric acid in basic slag has become obsolete in European countries.

During the past year the writer has had experience with the Wagner method of determining available phosphoric acid in basic slag and the valuations of this material that appear in our fertilizer bulletin have been based on this method.

The Wagner method as used in foreign countries has shown results agreeing closely with those obtained in field trials. It is as follows:

Weigh 5 grams of the slag and transfer it to a half-liter, bottle-shaped flask containing 5 cc of alcohol to prevent the slag from adhering to the flask. Make up to the mark with a 2 per cent citric acid solution at 17.5° C. The flask is fitted with a rubber stopper and put at once into a rotary apparatus for thirty minutes, making thirty to forty revolutions per minute. At the end of a half hour the solution is immediately filtered and the phosphoric acid is determined in an aliquot part of the clear solution by means of molybdic solution in the usual manner.

The analysis of two samples of slag by this method at the Massachusetts experiment station shows the following close agreement. No. 1, available phosphoric acid, 15.42 and 15.38; No. 2, 15.81 and 15.75.

In case of a bona fide sample of basic slag the valuation based upon mechanical analysis by use of a 100-mesh sieve agrees closely with the valuation based on the availability of the phosphoric acid by the Wagner method. In case, however, of a sample of slag adulterated with the natural mineral phosphate, the valuation based on mechanical fineness is obviously open to severe criticism. I think this question of sufficient importance to warrant a motion that I would herewith make, that the referee on phosphoric acid be instructed to make a study of the Wagner method of analysis with samples of basic slag and natural mineral phosphates, with a view to its adoption as an official method for the determination of available phosphoric acid in basic slag.

The papers by Mr. Lindsey and Mr. Haskins relating to the valuation of phosphoric acid in basic slag were referred to Committee A for action on recommendations contained therein.

## REPORT ON DAIRY PRODUCTS.

J. M. BARTLETT, *Referee.*

According to instructions given by vote of the association last year the referee has continued the study of analytical methods for condensed milks. The results reported at the last meeting indicated that the analysis of the sweetened product presented much greater difficulties than the unsweetened, particularly in the determination of fat; therefore, the referee decided to confine the work to one brand only, the sweetened milk. Twenty-six analysts signified a desire to cooperate, but not all of them were official chemists, many being commercial chemists more or less directly interested in food analysis.

### SAMPLES OF MILK.

On about April 1, a can of sweetened condensed milk together with a copy of instructions was sent to each chemist requesting the same. It was first intended to get a quantity of milk in bulk, thoroughly mix it in the laboratory, and send out the samples

in bottles to insure uniformity, but a letter from the Borden Company, who furnished the samples, describing their process, whereby the milk is continuously agitated until it reaches the cans, convinced the writer that all cans from the same batch must be as uniform as it is possible to make them.

#### INSTRUCTIONS FOR ANALYSIS OF CONDENSED MILK.

##### *Preparation of sample.*

Mix thoroughly by transferring the contents of a can to some dish sufficiently large to thoroughly stir and make the whole homogeneous. Weigh 40 grams into a 100 cc flask and make up to the mark with water.

##### *Total solids.*

*Method A.*—Dilute a measured portion of the above 40 per cent solution with an equal amount of water. Use 5 cc of the diluted mixture and proceed as in the case of milk analysis according to the method given in Bulletin 107, page 117, Method I, drying either on sand or asbestos fiber.

*Method B.*—Use Leach's method which is as follows: Dilute a portion of the 40 per cent solution with an equal amount of water and take enough of this solution to represent 1 gram of the condensed milk. Put in a tared platinum dish which will hold at least 25 cc and still further dilute with water until the dish is nearly full, rinsing the pipette into the dish, then allow the dish to remain in contact with live steam for at least two hours after the last traces of the water have apparently been evaporated, then transfer to the drying oven for a few minutes, cool in the desiccator and weigh.

##### *Ash.*

Ignite the residue from total solids, cool and weigh in the usual manner.

##### *Protein.*

Determine nitrogen by Kjeldahl or Gunning method in 5 cc of the 40 per cent solution and multiply by 6.25.

##### *Lactose.*

Dilute 50 cc of the 40 per cent solution in a 250 cc flask to about 200 cc, add 6 cc of Fehling's copper sulphate solution, and make up to the mark, filter through a dry filter and determine lactose as follows:

Take 25 cc each of the copper sulphate and alkaline tartrate solution, add 50 cc water and bring to boiling, then add 25 cc of the filtered milk solution and boil two minutes (by longer boiling the sucrose appears to throw down some copper), remove from the lamp and allow to settle one or two minutes then filter on a gooch crucible in the usual manner, weighing the cuprous oxid after drying at 100 degrees. Give weights of cuprous oxid found as well as percentages of lactose so methods of calculation can be compared. Also, if possible, determine lactose by polariscope in this solution.

##### *Sucrose.*

Place 25 cc of the above solution, used to determine lactose, in a 100 cc flask. Add 50 cc of water and 0.75 of a gram of citric acid and heat on the steam bath for thirty minutes, nearly neutralize with sodium hydroxid and determine total sugars in 25 cc with Fehling's solution in the usual manner, giving the weight of cuprous oxid as well as the percentages of sucrose. Also determine sucrose by difference, subtracting the lactose, protein, fat and ash from total solids.

##### *Fat.*

*Method A.*—Determine by double extraction method. (See Bureau of Chemistry, Circular 32, p. 6.)

*Method B.*—By the Babcock centrifugal method using the modification given in Bulletin 107, page 123.

*Method C.*—The Gottlieb method, the directions for which are as follows: Ten cubic centimeters of milk are measured into a glass cylinder three-fourths inch in diameter and about 14 inches long (see Landw. Vers. Sta., 40:6; a 100 cc burette or a eudiometer tube will do); 1 cc of concentrated ammonia is added and mixed well with the milk. The following chemicals are next added, in the order given: 10 cc of 92 per cent alcohol, 25 cc of washed ether, and 25 cc petroleum ether (boiling point

below 80° C.), the cylinder being closed with a moistened cork stopper and the contents shaken several times after the addition of each. The cylinder is then left standing for six hours or more. The clear fat solution is next pipetted off into a small weighed flask by means of a siphon drawn to a fine point (fig. 6, loc. cit.), which is lowered into the fat solution to within 0.5 cm of the turbid bottom layer. After evaporating the ether solution in a hood, the flasks are dried in a steam oven for two or three hours and weighed. This method is applicable to new milk, skim milk, buttermilk, whey, cream, cheese, condensed milk, and milk powder, but has been found of special value for determining fat in skim milk, buttermilk, cheese, and condensed milk. In the case of products high in fat, a second treatment with 10 cc each of ether and petroleum ether is advisable in order to recover the last trace of fat.

Chemists are requested to make at least two determinations by the methods given. On account of the quite large variations in the results reported by the chemists last year, the referee is very anxious to determine whether the differences are due to the inaccuracy of the methods or to the manner in which they are handled by different men. Everyone who has had much experience in making sugar determinations realizes how easy it is to get quite large variations in results by varying the method slightly.

If methods materially different from those given above are being used by anyone taking part in the work for the determination of sugars or fat in condensed milks, the referee will be glad to have results by such methods reported.

J. M. BARTLETT,  
*Referee.*

L. G. MICHAELS,  
*Associate referee.*

Eleven different chemists reported on the samples sent them and their results are given in the following table, together with some obtained at the Maine station:

*Cooperative work on samples of sweetened condensed milk (percentage results).*

Analyst.	Solids.		Ash.	Protein N×6.25.	Lactose.	Sucrose.			Fat.		
	Dried on sand or asbestos.	Leach's method.				Direct.	By difference.	Polariscope.	Double extraction.	Modified Babcock.	Gottlieb.
W. A. Brennon, Wisconsin station.....	72.44	72.08	1.81	.....	11.47	44.76	.....	.....	8.10	8.32	8.64
	73.14	73.19	1.82	.....	11.56	44.50	.....	.....	8.24	8.10	8.04
	73.06	72.90	1.66	.....	11.56	.....	.....	.....	.....	.....	.....
Average.....	72.89	72.72	1.76	7.42	11.53	44.68	44.01	48.51	8.17	8.21	8.34
E. M. Bailey, Connecticut station.....	72.25	73.71	.....	.....	.....	.....	.....	.....	8.00	8.25	.....
	72.06	73.50	.....	.....	.....	.....	.....	.....	7.80	8.40	.....
Average.....	72.15	73.60	.....	.....	.....	.....	.....	.....	7.90	8.33	.....
J. M. Bartlett, Maine station.....	70.70	.....	.....	.....	11.3	.....	.....	.....	.....	8.50	8.7
	70.50	.....	.....	.....	11.1	.....	.....	.....	.....	8.70	8.5
Average.....	70.60	.....	1.70	7.20	11.2	.....	43.24	.....	8.66	8.60	8.6
Sidney Davis, department of health, New York City.....	.....	73.13	1.72	6.96	11.72	.....	.....	44.09	9.00	.....	.....
L. W. Fetzer, Maryland station.....	72.01	74.47	1.53	7.20	11.42	.....	.....	.....	8.66	7.80	.....
	72.03	74.70	1.52	7.23	11.42	.....	.....	.....	8.63	7.95	.....
Average.....	72.02	74.53	1.53	7.22	11.42	37.85	44.20	.....	8.65	7.90	.....
H. B. Holland, Massachusetts station: Average of 4 tests.....	71.52	.....	1.66	7.42	.....	.....	.....	.....	7.29	.....	.....
Second sample, average of 3 tests.....	71.41	.....	.....	.....	.....	.....	.....	.....	7.85	.....	.....
C. H. Jones, Vermont station: Average of 2 tests.....	69.75	70.58	1.68	7.09	11.63	43.77	41.19	.....	8.16	8.10	.....
Second sample, average of 2 tests.....	72.20	.....	.....	.....	.....	.....	.....	.....	8.56	8.55	.....
C. P. Moat, Vermont board of health.....	70.00	72.51	1.51	.....	.....	.....	.....	.....	.....	6.6	.....

<sup>a</sup> Omitted from average.

<sup>b</sup> Dried on sand and extracted once.

<sup>c</sup> Extracted twice.



## Cooperative work on samples of sweetened condensed milk (percentage results)—Cont'd.

Analyst.	Solids.		Ash.	Protein N $\times 6.25$ .	Lactose.	Sucrose.			Fat.		
	Dried on sand or asbestos.	Leach's method.				Direct.	By difference.	Polariscope.	Double extraction.	Modified Babcock.	Gottlieb.
C. B. Morrison, Connecticut station (New Haven).....	68.75	70.55	1.95	7.05	.....	.....	.....	.....	8.19	7.2	.....
	68.97	70.81	2.05	7.06	.....	.....	.....	.....	8.04	7.5	7.98
Average.....	68.86	70.68	2.00	7.06	.....	.....	.....	.....	8.12	7.35	.....
A. J. Patten, Michigan station	72.35	73.87	1.80	7.06	12.05	.....	.....	.....	.....	7.20	7.96
	72.37	73.88	1.82	7.06	11.90	.....	.....	.....	7.01	7.20	7.80
Average.....	72.36	73.88	1.81	7.06	11.95	.....	44.63	43.65	7.01	7.20	7.88
Nelson & Landers, Binghamton, N. Y.....	72.45	.....	2.47	7.87	10.30	43.70	44.59	.....	8.52	8.40	8.30
A. C. Whittier, Maine station.	70.70	73.20	1.73	.....	11.02	.....	.....	.....	8.54	8.70	7.89
	70.80	73.33	1.72	.....	11.06	.....	.....	.....	8.45	8.70	7.78
Average.....	70.75	73.27	1.71	7.19	11.04	.....	44.79	44.79	8.50	8.70	7.83
A. D. Meeds, Minneapolis, Minn.....	72.67	73.20	1.74	6.50	9.78	43.07	46.64	.....	8.04	7.20	8.25
	72.80	73.00	1.74	6.50	9.74	43.06	46.74	.....	8.05	7.20	8.11
Average.....	72.74	73.10	1.74	6.50	9.76	43.07	46.68	.....	8.05	7.20	8.18
J. C. Colcord, Maine station..	70.80	.....	.....	.....	.....	.....	.....	.....	8.57	.....	.....
Average.....	71.57	72.80	1.69	7.25	11.35	43.80	44.12	44.18	8.17	7.87	8.16

\* Omitted from average.

## COMMENTS OF ANALYSTS.

*W. A. Brennon, Wisconsin station:* The solids were determined in Method A by drying on sand five and a half hours and in B by drying three hours on steam bath and two hours in hot-air oven.

*E. M. Bailey, Connecticut station:* Total solids were determined in Method A by drying on sand in an 8 cm aluminum dish, there being enough sand to make a total of 50 grams weight. Evaporation was first made on live steam, with frequent stirring. The dish was then wiped dry and put in a drying oven at 100° for thirty minutes, cooled and weighed, then again heated three hours, and for seven hours.

Per cent.

Average for thirty minutes.....	72.74
Average for three hours.....	72.40
Average for seven hours.....	72.11

In Method B the manipulation was the same as in A, except that no absorbent was used. Average for heating thirty minutes was 73.58 per cent; for two hours, 73.60 per cent.

In the double-extraction method for fat the first and second extractions were made for eight hours each and the third for four hours. In the centrifugal method the pipette was rinsed into the bottle.

*Lewis W. Felzer, Maryland station:* The methods involving dilution of a 40 per cent solution are very prone to cause an error, as it is next to impossible to dilute with a pipette or cylinder. Where this was directed I made a separate 20 per cent solution. The per cent of sucrose is better determined by difference, as the galactose which is formed by inverting the milk sugar evidently has a different reducing capacity from dextrose and levulose. In the case of the Leach-Babcock test for fat one can readily see that errors can creep in while drawing off the supernatant fluid in three instances.

*E. B. Holland, Massachusetts station:* Solution: A 20 per cent solution was prepared by diluting 100 grams of the milk to 500 cc. There was some separation of curd or fat, presumably the latter, which must have vitiated the results to some extent.

Moisture: Several aliquots of 5 and 10 cc were evaporated on quartz sand in a flat-bottomed dish at a low temperature until the bulk of the water was expelled, then

dried in an electric oven for two hours at 100° C. or in a vacuum oven below 70° C. (gauge reading, 29 inches).

Evaporation in a flat-bottomed dish without sand yielded low results, probably due to a retention of moisture by the nitrogenous film which formed on the surface. Similar treatment in a vacuum oven gave higher results, but below that obtained on sand. These figures are not reported.

Ash: Twenty-five cc were evaporated in a platinum dish with 5 cc of concentrated nitric acid and burned to a white ash.

Protein: Ten cc were treated by the Kjeldahl-Gunning method.

Fat: Our work of last season indicated that a single extraction gave higher results than the double and saved time and work. The dried solids on the sand were pulverized, washed with water, dried, and extracted with dry ethyl ether in a continuous extractor. Long heating of the residue containing fat (at 100° C.) should be avoided, as it appears to reduce the amount of fat that can be extracted.

*C. H. Jones, Vermont station:* The result reported on fat by the modified Babcock method is the average of ten determinations on three distinct 40 per cent solutions. The individual readings were 2.60, 2.65, 2.80, and seven readings of 2.70. Lactose determinations were made on distinct portions of the original sample. In the sucrose determination it was necessary to dilute the solution after inversion to 200 cc in order to have an excess of copper in the Fehling solution.

The result reported by the Leach method is the average of two determinations, 70.70 and 70.58 per cent, respectively. The platinum dish used did not have an absolutely flat bottom, but it was the nearest approach to anything of the kind available. I am at present unable to explain the difference obtained by the two methods, unless a too complete drying and consequent breaking down is obtained with Method A.

The result reported on the double-extraction method for fat is the average of two determinations from distinct solutions. They were, respectively, 8.20 and 8.12 per cent. The only awkward feature is the size of the filter paper used. The following modification of the Babcock method described was suggested by the use of the hardened filter on other laboratory determinations:

Method: Place 15 cc of the 40 per cent solution (6 grams) in a small-lipped beaker, diameter 1.5 inches, height 2 inches. Dilute with an equal amount of water; add 4 cc Fehling's copper solution; stir with glass rod. Filter through a 12.5 cm C. S. and S. 575 hardened filter. Wash thoroughly with water; stir on the filter with glass rod (100 cc is usually enough, though 160 cc had no lowering effect on the result). Return precipitate to original beaker, removing any remaining particles by washing with hot water through a fine-jet wash bottle. (The bulk is easily kept below 17 cc.) Stir with a glass rod. Pour into Babcock milk bottle. Add a portion of the acid to the residue in the beaker. Mix thoroughly, using stirring rod. Transfer to Babcock bottle. Repeat with remainder of acid. *Shake milk bottle thoroughly*, and then rinse beaker with a little hot water from the wash bottle and put into test bottle. Run as usual. The individual results obtained by this procedure were 2.70 for five readings and 2.65 on the sixth, three different 40 per cent solutions used.

I find it desirable, both with this method and with Method B, to use rather more sulphuric acid than is specified; often 18.5 cc. While the results by this procedure are not different from those obtained by Method B, yet when a number of samples are run a considerable gain is made in actual working time.

#### DISCUSSION OF RESULTS.

The results obtained this year are quite satisfactory on all determinations except fat. The lactose results, with three exceptions, are probably as good as one could hope to get from a number of chemists working independently and not making a specialty of sugar determinations. There are some variations in total solids for which it seems difficult to account. One might think it due to variations in the different cans of milk were it not for the fact that in some cases when the solids were as much as 2 per cent low, the other determinations, such as proteids, ash, etc., were as high or higher than the average. The referee can only account for these discrepancies in one of two ways, faulty sampling, or that the sugars were allowed to ferment and cause loss before the determinations were made. It is believed that all determinations should be made as soon as the solutions are made up, and no solution which has stood in a warm laboratory twenty-four hours should be used for the determinations of solids or sugars. Leach's method appears to give high results, probably because of the large amount of sugar present to hold the water.

The ash results are for the most part very good and concordant, with the exception of three, which were probably burned down hard without leaching. It is very evident that leaching with hot water after thorough charring is necessary in the presence of sucrose.

The sucrose results are probably as good as one could expect to obtain, but inasmuch as this is not a normal but an added constituent of the milk it is best determined by difference.

The results on fat, one of the most important constituents of the milk, are far from being satisfactory. The referee believes that these discrepancies are due to three causes. First, lack of experience with this kind of material. Second, a lack of detail in giving the double-extraction method in Circular 32, and, third, faulty instructions in directing a 40 per cent solution to be used for this method. This solution, as shown by the tables below, is too concentrated to get the best results when as large an amount as 40 per cent cane sugar is present. This degree of concentration makes such a thick layer of sugar on the paper coil that quite a large proportion of the fat is left on the paper after the first extraction, and then soaking in water causes a mechanical loss of fat when the sugar is dissolved off. Such loss was proven by examination of the slight scum rising on the water, which, under the microscope, showed the presence of fat globules. When the work is carefully done, however, and dry ether is used, this loss is only small, amounting to one or two tenths of 1 per cent. Our results show that as high results can be obtained with 1 gram in a 10 or 20 per cent solution in a single extraction of fourteen hours as with a more concentrated 40 per cent solution and a double extraction. The highest and most concordant results, however, were obtained with a 10 or 20 per cent solution and double extraction.

Mr. Geisler, the originator of the double-extraction method, stated, under date of January, 1908, that he had no changes to suggest from those given in his original paper published in the *Journal of the American Chemical Society* in 1900 except that the time of each extraction should be extended to seven or eight hours instead of four or five and that strictly dry ether probably is the best solvent on account of its constant boiling point. In Mr. Geisler's paper he emphasizes the fact that the milk should be evenly distributed over the surface of the paper coil; also that the ether should be anhydrous to prevent the paper coil from becoming soggy. The method of drying on asbestos or paper in tubes is not so desirable as on strips of paper on account of the long heating required to dry out completely.

The modified Babcock method, in the hands of men who have had much experience with it, usually gives very good results, but it is not to be considered as accurate as the gravimetric, and as the reading is multiplied by three every error is increased threefold. When the milk was fresh we were able to get very clear separations with the copper sulphate in the centrifuge, and the sugar solution could be easily decanted without loss of fat, the curd and fat remaining in a hard mass at the bottom of the bottle, but in testing some cans later, which had stood in the laboratory for three or four months, the separation was not so complete, and it was necessary to pass the solution through a filter, washing the particles of curd back into the bottle to obtain all the fat, and in several instances the results were low. This operation made the method somewhat longer and more tedious.

The results reported by the Gottlieb method are not very satisfactory and the referee found but little time to test it. Only two tests were made and they gave by direct weight over 9 per cent fat, but it was found that some proteid or foreign matter was clinging to the bottom of the flasks after the fat was dissolved out with hot benzene. After weighing and deducing this weight from the original the results agreed very closely with the gravimetric. The writer believes that by observing proper precautions this method can be made very reliable, particularly for sweetened condensed milk. Small separatory funnels are more desirable than the long tubes recommended in the method.

Some figures obtained in the Maine laboratory are given in the following table, and lead the writer to believe that good results can be obtained when the double-extraction method is carried out as follows:

Prepare strips of soft white filter paper 4 by 24 inches of the quality of the S. & S. No. 597, by soaking two or three hours in alcohol, then after thoroughly drying in the oven extracting several hours with ether until no residue is left from the ether as it comes through. Then take 10 cc of a 20 per cent solution of the condensed milk and distribute it carefully over the whole surface of the thoroughly dried paper. This is best done by attaching one end of the paper to some object and holding the other end out straight so that the pipette can be emptied by passing the point back and forth over the whole surface. To dry the paper, suspend it over a copper wire in the drying oven, where it will thoroughly dry out in two hours or much more rapidly than if coiled up or put in a tube. After drying roll up in a coil, wind with thread or small copper wire, place in the extractor, and extract not less than eight hours. If it is the sweetened product remove the coils from the extractor, loosen the wire or thread, dry and suspend in 500 cc of water for two hours, then return the coils to the oven and dry as before, and extract again for not less than five hours. Five cc of milk and a coil 4 by 12 inches may be used if preferred.

*Determination of fat in condensed milk by modifications of the double-extraction method.*

Modifications.	Fat.
	<i>Per cent.</i>
5 cc (2 grams) of 40 per cent solution extracted 8 hours with ether:	
Exhausted with water and extracted 5 hours more (average of 4 samples).....	8.28
Exhausted a second time and extracted a third time.....	8.45
cc (2 grams) of a 20 per cent solution on a coil 5 by 24 inches:	
10 cc (2 grams) of a 20 per cent solution.....	8.55
Exhausted with water and extracted 5 hours more.....	8.78
cc (2 grams) of a 20 per cent solution on a coil 5 by 24 inches:	
Extracted 14 hours.....	8.42
Exhausted with water and extracted again 6 hours.....	8.69
5 cc (1 gram) of a 20 per cent solution on a coil 5 by 12 inches:	
Extracted 5 hours.....	8.45
Exhausted with water and extracted 5 hours more.....	8.78
5 cc (1 gram) of a 20 per cent solution on a coil 5 by 12 inches:	
Extracted 10 hours with ether.....	8.36
Exhausted with water and extracted again.....	8.85
10 cc (1 gram) of a 10 per cent solution on a coil 5 by 24 inches:	
Extracted with ether 14 hours.....	8.42
Exhausted with water and extracted 5 hours more.....	8.95

### RECOMMENDATIONS.

It is recommended that—

(1) The following methods be adopted as official methods:

#### 1. PREPARATION OF SAMPLE.

Place the can, if cold, in water at 30° to 35° C. until warm. Open and mix thoroughly by transferring the contents of the can to some dish sufficiently large to thoroughly stir and make the whole mass homogeneous. Care must be taken to scrape out all milk adhering to the interior of the can. Weigh 100 grams into a 500 cc flask and make up to the mark with water. If the milk will not completely dissolve, each portion must be weighed out separately for analysis.

#### 2. TOTAL SOLIDS.

Use 10 cc of the above 20 per cent solution and proceed as in the case of milk analysis according to the method given in Bulletin 107, page 117, Method I, drying either on sand or asbestos fiber.

#### 3. ASH.

Ignite the residue from 10 cc of the 20 per cent solution at low red heat, leach with hot water if sucrose is present, ignite the residue and filter until white, add the leachings, evaporate to dryness again in usual manner and weigh.

## 4. PROTEIN.

Determine nitrogen by Kjeldahl or Gunning method in 10 cc of the 20 per cent solution and multiply by 6.38.

## 5. LACTOSE.

Dilute 100 cc of the 20 per cent solution in a 250 cc flask to about 200 cc; add 6 cc of Fehling's copper sulphate solution and make up to the mark; filter through a dry filter and determine lactose by the Walker method, boiling only two minutes with the Fehling solution.

(2) The methods for determining sucrose and fat be given further study.

## REPORT ON FOODS AND FEEDING STUFFS.

By FRED W. MORSE, *Associate Referee.*

The request to serve as referee on cattle foods was unexpected and found me unfamiliar with the most recent work of the association on this class of materials. Noting that it was recommended last year to continue the trial of the methyl pentosan determination after the method of Ellett, an attempt was made to simplify the method before asking for cooperation from other members. Wholly satisfactory results have not yet been obtained, but it seems possible, with a little more time, to accomplish such a modification.

It was also planned to compare the effects of the use of Ellett's method on some standard cattle food alongside of a substance known to contain methyl pentosan. For the latter there was accessible plenty of the seaweed, *Fucus vesiculosus* or rockweed, and a quantity was obtained, dried, and pulverized. For the standard cattle food, wheat bran was selected, since its content of pentosan is good, and Widsøe reported no evidence of methyl furfural in it by the qualitative tests.

The method of procedure was to follow the provisional method for pentosan determination throughout and, after weighing the precipitated phloroglucid, to extract with alcohol by Ellett's method of digesting the crucible and contents in a small quantity of alcohol at 65° C., filtering, and repeating the operation until the filtrate finally becomes colorless. A marked solubility of the precipitate was observed in both cases. This was unexpected in the bran, and considerable time was spent in repeating determinations. Results on bran varied much; but the seaweed gave reasonably concordant figures. By this time it was too late to send out samples to other chemists. Another point was noticed in the prosecution of the work, namely, that the provisional method for pentosans seldom if ever yielded furfural-free distillates when the prescribed limit of volume was reached. The drops would still show traces of furfural.

These points of disagreement from published matter about the different forms of pentosans have convinced the referee that more work is needed on this provisional method in some of the details. There is an important field for research in our common coarse fodders and the concentrated by-products in working out the constituents of the nitrogen-free extract. Most of the methods now in use are difficult of manipulation and more or less approximate in their results. Comparatively little attention is paid to them, since the conventional methods of fodder analysis answer the practical feeder's purpose.

Nevertheless, progress in nutrition studies demands more attention to the less-known carbohydrates, since their digestibility and consequent food value are unknown quantities.

The referee has no recommendations to make; but if no instructions are received from the association it is his intention to continue the study of these newest methods of determining the less-known carbohydrates.

*Ellett's method applied to Fucus vesiculosus.*

[Two grams of material.]

Total phloroglucid.	Loss by extraction with alcohol.
<i>Gram.</i>	<i>Gram.</i>
0.2403	0.0429
.2467	.0516
.2497	.0341
.2405	.0434
.2664	.0446
Mean .2487	.0433

## BEST RESULTS BY WASHING WITH HOT ALCOHOL.

<i>Gram.</i>	<i>Gram.</i>
0.2366	0.0407
.2533	.0366

## THE DETERMINATION OF ACIDITY IN CATTLE FEEDS.

By JOHN PHILLIPS STREET, *Referee.*

The acidity of a cattle food is due to the presence of hydrogen ions. In a solution containing a mixture of salts of organic and inorganic acids it makes practically no difference whether this acidity was originally produced by the addition of a small amount of an organic or of an inorganic acid, for the final result is essentially the same; that is, the presence of a certain proportion of free hydrogen ions and of the ions of all the various salts which are present in the solution. The question of acidity, therefore, is one of degree rather than of kind and, from a physiological view point, depends on the nature of the salts which are present in the solution under consideration. Let us take an example. We have a solution containing sodium chlorid and sodium acetate. In this we have sodium ions, chlorin ions, acetate ions, undissociated sodium acetate molecules, and undissociated sodium chlorid molecules. If a small quantity of hydrochloric acid is added to this solution it will then contain, in addition to the substances above named, a certain quantity of hydrogen ions and a correspondingly greater quantity of chlorin ions. If a molecularly equivalent quantity of acetic acid is added instead of hydrochloric, the solution will contain the hydrogen ions, as in the first case, and the number of acetate ions will be correspondingly increased.

If we now measure the acidity of each of these two solutions with phenolphthalein as an indicator, the result will be the same, for this indicator gives a pretty accurate measure of free and potentially free hydrogen ions. If we measure the acidity of these solutions by means of delicate litmus paper the degree of acidity will be found to be less than that as determined by phenolphthalein. The reason for this is to be found in the fact that litmus is a relatively stronger acid than phenolphthalein and reacts with the base before all the acid hydrogen of the acetic acid has been acted on. The effect of the presence of organic salts is to reduce the number of free hydrogen ions, in comparison with that which would be present in a solution to which had been added the same quantity of mineral acid in the presence simply of inorganic salts of strong bases with strong acids, such as sodium chlorid or sodium sulphate, and it is also clear that it is not possible to determine in a solution containing a mixture of organic and inorganic salts, which show an acid reaction, whether this reaction was originally caused by the action of a mineral acid or of an organic acid. The indicators that are commonly supposed to distinguish between mineral and organic acids in mixtures

containing salts of weak bases and strong acids, or of strong bases and weak acids, give entirely different results from those obtained in solutions free from such salts, for by hydrolytic dissociation these salts contribute to the solution a certain quantity of hydrogen or hydroxyl ions, according to the nature of the salts present and the concentration of the solution, which ions exert an effect on the indicator in one direction or the other.

In a mixture of weak and strong acids and their salts, phenolphthalein, which is the weakest indicator commonly employed, gives the total amount of acids present stronger than phenolphthalein, itself an exceedingly weak acid. If a stronger acid indicator, e. g., litmus, is in the above mixture, it will appear to have less total acid, because the litmus itself reacts with the base before the weaker acids are acted on.

It is thus clear that, for such a mixture, it is impossible to determine the acidity of its solution, and, furthermore, it is not even possible by titration to determine the actual concentration in free hydrogen ions, which, from a physiological standpoint, is the true question under consideration.

It has been the practice of physical chemists to determine the concentration in hydrogen ions by inverting cane sugar, a process which closely corresponds with the enzym reactions of physiological processes.

The acidity of a cattle feed may come from a mineral acid used in its preparation, from organic acids natural to the product itself, or developed by fermentation during its preparation, and possibly, in some cases, from phosphates having an acid reaction and normally present in the feed.

Jordan's studies (Bulletin 238, Geneva station), however, indicate that "our commercial feeds of vegetable origin do not contain appreciable quantities of phosphorus in inorganic combination."

Ensilage is an example of a feed containing considerable amounts of organic acids developed in the silo by fermentation. A number of other feeds which are by-products of manufacturing processes contain organic acids resulting, likewise, from fermentations taking place during manufacture.

In view of the fact that the presence of free mineral acids in certain feeds has been suspected or affirmed, I wish to raise the question of the methods involved and ask the association to make it the subject of inquiry, in order that an accurate method of testing for acidity may be found and adopted. As matters now stand, we are depending wholly on volumetric methods and the use of indicators, and the question to be settled first of all is, Just what do indicators indicate?

Too little attention has been given to the acidity due to the proteins themselves and their varying action with different indicators. Osborne <sup>a</sup> has pointed out that the proteins are not neutral bodies, like the carbohydrates, and that the general assumption that a solution containing protein matter, and showing neither acid nor alkaline with litmus, is chemically neutral, is erroneous. Many experiments have shown that certain protein solutions, when neutral to litmus, are acid to phenolphthalein and alkaline to lacmoid. It has also been established that a notable amount of acid can be added to a protein solution before an acid reaction with tropaeolin, alizarin, or phloroglucin and vanillin is given.

As Osborne says, <sup>b</sup> it is of importance "to know whether litmus can be used to determine the point when all combined acid has been converted into neutral salts of potassium or sodium and all the protein substance has been set free, or whether, as we know is the case, when tropaeolin or lacmoid is used as an indicator, more acid still remains combined."

Aqueous solutions of crystallized ovalbumin, solutions in sodium chlorid brine of excelsin, amandin, vignin, conglutin, glycinin, corylin, phaseolin, and legumin, and solutions of zein, gliadin, and hordein in 75 to 90 per cent alcohol, which were either

<sup>a</sup> J. Amer. Chem. Soc., 1902, 24: 39.

<sup>b</sup> Loc. cit.

neutral or acid to sensitive neutral litmus paper, when made neutral to litmus were, in every case, still acid toward phenolphthalein.

Osborne further says:

To render gram portions of these several protein preparations neutral to litmus required in a few cases not any, in most cases from 0.1 to 1.5 cc of decinormal alkali; while to make the same gram portions neutral to phenolphthalein required the further addition of from 0.7 to 1 cc of decinormal alkali, except for legumin, which required 2 cc. Edestin made neutral to phenolphthalein and dissolved in sodium chlorid solution reacts distinctly alkaline toward litmus. This alkaline reaction is caused by the edestin itself and not by organic salts of the alkali, since such preparations yield a very small amount of ash, less than 0.05 per cent, which is neutral to both litmus and phenolphthalein. \* \* \* Solutions of all the other protein bodies I have examined, when similarly made neutral to phenolphthalein, react decidedly alkaline with litmus.

In the investigation which the referee reports at this time no attempt was made to determine total acidity, only those acids being taken into account which were extracted by a rather prolonged treatment with water. Sixteen samples of gluten feed, representing five brands, two of wheat bran and one each of wheat middlings, wheat feed and cottonseed meal, were examined.

Ten grams of the feed were weighed into a beaker and stirred with 50 cc of water for ten minutes, then transferred to a plain wet filter and washed with successive small portions of water, until the washings amounted to 150 cc; the extract was then made up to 200 cc with water and 20 cc portions (=1 gram feed) used in the subsequent titrations. A blank determination was also made with 200 cc of water run through a filter paper as before, and the washings were found to be neutral to methyl orange, phenolphthalein, and litmus.

The following indicators were used:

*Phenolphthalein:* One gram dissolved in 100 cc of 50 per cent alcohol.

*Litmus paper:* Very sensitive neutral paper.

*Methyl orange:* One gram dissolved in 1,000 cc of water.

*Congo red:* One gram dissolved in 100 cc of 30 per cent alcohol.

*Günzburg's reagent:* Two grams of phloroglucin and 1 gram of vanillin dissolved in 30 grams of alcohol.

*Toepfer's reagent:* One per cent solution of phenolphthalein in alcohol and 0.5 per cent solution of dimethylamidoazobenzol.

The alkali used was approximately decinormal sodium hydroxid, 1 cc being equal to 0.003996 gram sodium hydroxid.

Twenty cubic centimeter portions of the watery extract, equal to 1 gram of feed, were taken for each test. Owing to the usually highly colored solutions, the aliquot was diluted with 500 cc of water for the test with methyl orange, and with 50 cc for the other indicators, except in the Günzburg test, where 0.5 cc of the extract and the same quantity of the reagent were used. Three hydrochloric acid solutions were also prepared, N/14, N/28, and N/56, respectively.

The Günzburg and Toepfer tests and Congo Red are recommended as reliable in determining whether or not free mineral acid is present. These tests were applied first to the three hydrochloric acid solutions and unmistakable positive results were secured with all, the most dilute acid used, N/56, equivalent to about 0.065 per cent of hydrochloric acid, responding perfectly to the reactions indicated. A mixture of one of the aqueous feed extracts and dilute hydrochloric acid, the total mixture containing 0.065 per cent of free hydrochloric acid, was likewise subjected to these tests and positive proof was secured that nothing present in the feed extract in any way interfered with the delicacy of the reactions. However, following the suggestions of Osborne's work, when the salt of a weak acid, for instance, sodium acetate, was added to these same test solutions, none of the above prescribed tests for free mineral acidity responded, although hydrochloric acid had been added in every case. This experiment shows quite conclusively the danger and inaccuracy of asserting either the presence or absence of mineral acids from data obtained by these tests.



None of the feed extracts showed any acidity to methyl orange, a result quite to be expected. Referring to methyl orange, Ostwald says <sup>a</sup> "should the acid be weak, i. e., only slightly ionizable (the ionization being still further reduced by the presence of the neutral salt formed in the liquid), the quantity of hydrogen ions on passing the point of neutralization is too small to allow of the formation of a visible amount of the nonionized molecules of methyl orange, and the red color only appears after a considerable excess has been added, and then only by degrees."

The conditions thus described by Ostwald seem to be identical with what we have in these feed extracts. Five samples of Buffalo gluten feed required from 1.10 to 1.80 cc of decinormal alkali to neutralize the acidity of 1 gram of feed, using litmus; from 2.55 to 3.40 cc, using phenolphthalein, and from 2.70 to 3.65 cc, using Toepfer's reagent.

Five samples of Globe gluten feed, with the same amount of feed, required from 1.35 to 1.90 cc with litmus; 2.95 to 3.80 cc with phenolphthalein and from 2.90 to 3.80 cc with Toepfer's reagent.

Cream of corn gluten feed and Pekin gluten feed gave corresponding results with the three indicators; while Douglas gluten feed showed the same relation, but a much lower acidity, 0.25 cc with litmus, 0.40 cc with phenolphthalein and 0.35 with Toepfer's reagent.

The samples of wheat bran, middlings, and feed required 0.50 to 1 cc with litmus, 0.90 to 1.70 with phenolphthalein and 1 to 1.80 with Toepfer's reagent.

Cottonseed meal required 0.65 cc, 1 cc and 1 cc, respectively, with the three indicators.

The average acidity of all the feeds was 1.18 cc with litmus, 2.37 cc with phenolphthalein and 2.50 cc with Toepfer's reagent.

These results correspond perfectly with what we should expect if the acidity came from dissociation of protein salts alone, or from salts of weak organic acids. I therefore urge the association to take up the matter of acidity of cattle feeds and consider how the results obtained by current methods can be applied to agricultural problems.

The appended table presents the determinations in detail.

*Acidity of gluten feeds.*

[In terms of 1 gram of feed.]

Number.	Brand.	Protein.	Phenolphthalein.		Litmus.		Toepfer test (dimethylamidoazobenzol and phenolphthalein).	
			Tenth-normal sodium hydroxid.	Equal to grams of sodium hydroxid.	Tenth-normal sodium hydroxid.	Equal to grams of sodium hydroxid.	Tenth-normal sodium hydroxid.	Equal to grams of sodium hydroxid.
		<i>Per cent.</i>	<i>cc.</i>		<i>cc.</i>		<i>cc.</i>	
21435	Buffalo	23.37	3.40	0.0136	1.80	0.0072	3.50	0.0140
21448	do	26.62	2.55	.0102	1.20	.0048	3.10	.0124
21454	do	26.06	3.10	.0124	1.60	.0064	3.65	.0146
21470	do	26.31	3.35	.0134	1.65	.0066	3.65	.0146
21548	do	25.00	2.70	.0108	1.10	.0044	2.70	.0108
21456	Cream of corn	24.75	2.60	.0104	1.20	.0048	3.00	.0120
21518	Douglas	20.12	.40	.0016	.25	.0010	.35	.0014
21416	Globe	25.87	3.55	.0142	1.85	.0074	3.80	.0152
21439	do	26.00	3.40	.0136	1.85	.0074	3.50	.0140
21490	do	26.94	3.80	.0152	1.90	.0076	3.70	.0148
21502	do	26.37	2.95	.0118	1.45	.0058	2.90	.0116
21549	do	25.06	3.40	.0136	1.35	.0054	3.45	.0138
21409	Pekin	26.75	2.85	.0114	1.70	.0068	3.20	.0128
21487	Brand unknown	28.19	3.65	.0146	1.60	.0064	3.80	.0152
21516	do	22.75	.50	.0020	.40	.0016	.70	.0028
21533	do	21.62	1.05	.0042	.60	.0024	1.30	.0052
21415	Wheat bran	15.31	1.00	.0040	.50	.0020	1.30	.0052
21458	do	14.81	1.00	.0040	.50	.0020	1.10	.0044
21423	Wheat middlings	18.06	1.70	.0068	1.00	.0040	1.80	.0072
21437	Wheat feed	15.94	.90	.0036	.60	.0024	1.00	.0040
21428	Cottonseed meal	45.06	1.00	.0040	.65	.0026	1.00	.0040

## THE MANUFACTURE OF GLUTEN FEED.

By T. B. WAGNER.

Among the concentrated feeding stuffs found on the American market we may concede to gluten feed the first place, not only because of the high percentage of nutritive materials in gluten feed, but because of its palatability and its remarkable degree of digestibility. Within the last few months various statements have appeared in chemical journals, as well as in bulletins issued by agricultural experiment stations, with reference to the chemical analysis of this product. Considering the importance of gluten feed as an animal food, anything published on the subject will be read with interest, not only by officials connected with agricultural experiment stations, but by dealers and buyers, and, last but not least, by the manufacturers themselves. In view of this general interest, it may not be amiss to state the details of its manufacture. Broadly speaking, gluten feed is the ground kernel of Indian corn, from which the germ and most of the starch have been removed. The following steps lead up to its production:

The corn bought by us is of the No. 2 and No. 3 grades. To remove impurities, stones, dirt, dust, etc., the grain is passed through cleaning and separating machinery and the purified corn is then delivered to the steeping tanks, wherein it is soaked in warm water, slightly acidulated with sulphur dioxide. This treatment brings about a softening of the grain and facilitates the subsequent separation of the germ, which is effected after it has passed through a preliminary grinding whereby the corn is broken up and the germ set free. The balance of the material is now ground fine in Buhr mills, the coarser part, namely, the bran, being separated by running the mass over silk sieves, while the starch liquor is concentrated and sent over slightly inclined planes, the "starch tables," upon which, by a process of settlement and washing, the starch fills up in a solid layer. The lighter ingredients, gluten, fiber, etc., are carried off in the current of water over the end of the starch tables. We have thus obtained, first, the germ from which the well-known corn oil and corn-oil cake are obtained; second, the starch which furnishes the raw material for the corn starch of commerce and the manufacture of corn sirup and corn sugar; third, the bran, being the hulls of the kernel; and, fourth, the gluten. The third and fourth, after repeated washings, are united, when still in a wet state, deprived of the largest part of the water by filter pressing, and delivered to the driers, where the water is reduced to approximately 10 per cent. The feed is now passed through grinding mills and reduced to a considerable degree of fineness.

The gluten feed thus obtained varies in composition in proportion to the efficiency factor prevailing in the individual works. For instance, in a well equipped and well regulated factory the amount of protein usually runs at 26 per cent (on a commercial basis), whereas in factories conducted less efficiently the amount of protein may not exceed 18 per cent. The amount of starch in the feed will vary correspondingly. I have made the statement before that gluten feed represents the corn minus germ and starch. You will ask, and very properly so, What becomes of the mineral constituents of the corn and the soluble organic matter, which are extremely valuable—as, for instance, the organic phosphorus compounds? By far the largest amount of these constituents is leached out in the steeping of the corn. Were it desired only to recover the phosphorus salts, there would not be much difficulty involved in isolating them, but the steep water contains a large amount of other ingredients which greatly add to the food value of the gluten feed, such as albuminoids, sugar and other carbohydrates, potassium salts, etc., which, however, are hygroscopic and frustrate all efforts to recover them in dry form. Dr. Arno Behr devised ways and means of recovering these substances, which are fully described in United States patent No. 491,234, issued February 7, 1893. Briefly explained, Behr recovers these constituents of the

corn by evaporating the steep water after careful treatment to a thick sirup, which contains these substances partly in solution and partly in suspension. This sirup is added to the feed, which latter forms an ideal absorbent.

An analysis of gluten feed thus prepared has the following average composition:

	Per cent.
Water.....	10.36
Protein.....	25.95
Fat.....	2.18
Starch.....	18.09
Fiber.....	6.50
Ash.....	3.70
Nitrogen-free substance (by difference) <sup>a</sup> .....	33.22
Soluble (approximate).....	15.50

From the process outlined above it is obvious that no extraneous matter is introduced into the feed and that the ingredients which go to make up the feed occur there in the same form as in the corn itself, although, of course, in a more concentrated form. It was not without surprise, therefore, that I noticed in a number of analyses published recently a reference to an "acidity" of the feed, which was reported as hydrochloric acid. It is not quite plain why the acidity was expressed in such a manner, as no hydrochloric acid, or for that matter any other mineral acid, is present, none having been introduced at any stage of the process of manufacture. It certainly would not occur to anyone to report the acidity in fruits, vegetables, cider, or wines as hydrochloric acid, no more than in the case of wheat flour—patent flour—in which the acidity runs nearly as high as some of the acidities reported in gluten feeds. I am not prepared to state at this moment with any degree of finality whether this apparent acidity is due to acid salts, such as the organic phosphorus compounds, or to the presence of a slight amount of lactic acid, or to proteid bodies, such as the acid albumins; but, whatever causes it may be due to, if the absence of free mineral acids has been proved, it should be reported as an organic acid, preferably lactic. It might perhaps be still better to state the number of cubic centimeters of normal alkali required to neutralize.

In this connection it is of import to note the varying results obtained in acidity determinations, depending upon the character of the indicator employed. To cite an instance, we have found that phenolphthalein causes the acidity to appear two to three times higher than rosolic acid. Again, when methyl orange is used, an alkalinity is indicated. These discrepancies and variations make it desirable—in fact, necessary—that the official methods governing the analyses of feeding stuffs provide a standard indicator for such acidity; means should also be provided for expressing properly such acidity as may be found in the feed.

If, as a safeguard, it is deemed advisable to test for free mineral acid, Toepfer's test (dimethylamidoazobenzol), or the even more delicate Günzburg test (phloroglucin), are to be recommended. These tests are generally employed in physiological research and reveal the slightest traces of free mineral acids in the presence of organic acids.

A great desideratum in gluten feed is uniformity; that is, the feed made at the different factories located in different sections of the country should be uniform in composition as well as in appearance. So far as the first is concerned, the variation is very slight, the processes employed in our various factories being under such control as to insure practically uniform composition, irrespective of point of manufacture.

The appearance of the feed is of considerable moment. In former years the corn delivered to our factories was mostly of the yellow type, the amount of white corn delivered being rather insignificant. During the past four years, however, the situa-

<sup>a</sup> Contains 17.18 per cent pentosans.

tion has been quite the reverse. The supply of corn is not within our control. We have accomplished uniformity in our feeds so far as protein, fat, and the other ingredients are concerned, and so far as the physical condition of the feed is involved, but we can not reach the same degree of uniformity as regards color so long as the selection of the corn is not within our power. Gluten feed obtained exclusively from yellow corn has a beautiful yellow color, whereas feed made from white corn has an uninviting grayish color, so that, depending upon the amount of yellow and white corn going through the process, the color of the resultant feed may vary from a golden yellow through all the hues down to a grayish white. You will recognize the difficulties connected with the marketing of a product which to-day may run yellow and a week from now white. Speaking from my own experience, this point was brought home to me very forcibly in 1904, when the white variety of corn predominated in our corn supply. The feed produced from such corn was uninviting in appearance. In a very short time dealers, particularly in the Eastern States, began to complain, stating that they were not receiving the old standard gluten feed which they had been familiar with for a long period of years. Our assurance that the feed was the same, that the amount of proteid matter was the same, that the feed value was the same, and that the feed was up to standard in every particular, except color, did not avail, and we were not only threatened with, but actually suffered, a considerable loss of business. We advised the trade fully of the existing conditions, emphasis being laid upon the fact that the color should not be the determining factor in fixing the intrinsic or commercial value of the feed. Feeders, however, refused to accept such explanations. It seemed impossible to convince them that a brand of feed, yellow one day and white the next, could have been made by the same methods and be the same feed in fact.

As a solution of this difficulty, it was suggested that wherever the feed ran "short," so far as color was concerned, that the feed be standardized by the addition of the requisite amount of artificial color, preference being given to naphthol yellow-S. The feeder readily accepted this changed condition. Although informed that the feed is artificially colored, he prefers to buy it that way. It is plain from the above that the manufacturer is not acting from choice when adding color to his feed, but he is forced to do so by a popular demand. The practice of standardizing the color of gluten feed is no different than that practiced by the farmer in coloring butter. June butter is his standard, and in adding color to the butter he aims at matching the natural color of June butter, because the consumer likes that particular color best. Thus the feed obtained exclusively from yellow corn is the standard for color, and when a factory receives only two-thirds or less of its supply in the form of yellow corn, sufficient coloring matter is added to match the feed obtained exclusively from yellow corn. It thus happens that at one of our factories, located in southern Illinois, we do not add at this time a grain of color to the feed, whereas in another factory, located in Iowa, color is added in approximately the same proportions as in the case of colored confectionery. In other words, the practice of standardizing the color of the feed is not a regular practice, but depends from day to day entirely upon the character of the corn supply.

As a matter of chemical interest I would like to call attention to the rapidity with which the gluten of the corn combines with azo colors, such as naphthol yellow-S, forming an insoluble lake. This combination is effected without the use of any mordant, acids, or similar agents and tends to prove the acid character of some of the proteid compounds.

## REPORT ON THE SEPARATION OF NITROGENOUS BODIES: MILK AND CHEESE PROTEIDS.

By L. L. VAN SLYKE, *Referee*.

The referee selected the following subjects for investigation:

(1) The acetic-acid-precipitation method for determining casein in milk, especially with reference to the following points:

- (a) The use of less acid.
- (b) The influence of acid on the redissolving of casein.
- (c) The effect of temperature on the solution of casein by acetic acid.
- (d) The effect of various preservatives on the accuracy of the acetic-acid method.

(2) The selection of an official method by the association for the determination of milk albumin.

(3) A simple, rapid, and accurate volumetric method for determining milk casein.

For the cooperative work of 1907-8 the referee selected the Matthaïopoulos volumetric method for the determination of casein. Results from only one of the cooperators was received. The method is as follows:

### SOLUTIONS REQUIRED.

- (1) An approximately twenty-fifth-normal solution of sulphuric acid.
- (2) A tenth-normal solution of sodium hydroxid.
- (3) A 1 per cent alcoholic solution of phenolphthalein.

### METHOD OF PROCEDURE.

Into each of two 200 cc beakers measure 20 cc of milk and 80 cc of water. Call one *A* and the other *B*. Into *A* let the twenty-fifth-normal sulphuric acid run drop by drop with constant stirring of the diluted milk until the casein precipitates in large flakes. After three to five minutes filter through a dry filter (S and S 589, 9 cm, recommended) and collect the filtrate in a graduated, dry, 100 cc flask. If the first portions of the filtrate are turbid, pour back on filter. If the filtrate continues turbid, not enough acid has been used to precipitate the casein completely; in which case take a fresh sample and add 0.2 or 0.3 cc more acid. The amount of acid required varies with different milks, ranging in the sample studied from about 23 to 27.5 cc. Any excess of acid must be avoided. Collect 100 cc of clear filtrate and transfer it to a beaker, rinsing the flask carefully, add 1 cc of the phenolphthalein solution and titrate to a pale pink color with tenth-normal sodium hydroxid. Note the number of cubic centimeters of alkali used.

Treat the contents of beaker *B* with twenty-fifth-normal sulphuric acid, using exactly the same amount as in the case of *A*. Add 1 cc of phenolphthalein solution and titrate to a pale pink with tenth-normal sodium hydroxid. Note the amount of alkali used.

The values obtained in *A* and *B* are then used in making the following calculations:

$$\left[ B - \frac{A(100+H)}{100} \right] \times 0.11315,$$

in which *B* is the amount of alkali used in titrating the mixture of water, milk, and twenty-fifth-normal sulphuric acid; *A* is the amount of alkali used in titrating the filtrate; *H* is the amount of twenty-fifth-normal sulphuric acid used in precipitating casein; 0.11315 is a constant factor based on the equivalent weight of casein as shown by its salts with bases. The results are then calculated from 20 to 100 cc.

Each cooperator was requested to apply this method to samples of fresh milk of his own selection and compare the results with those obtained by the official method.

*Determination of casein in milk.*

Analyst.	Official method.	Volumetric method.
L. W. Fetzer, Maryland station. . . .	2.54	2.73
	2.52	2.79
A. W. Bosworth, New York station. . . .	3.06	3.06
	3.06	3.06
	3.06	3.07
	3.06	3.06
	3.00	2.88
	3.03	2.90
		3.05

From the results thus far obtained, the method appears to be a promising one. It will probably require some modification to give uniform results.

## RECOMMENDATION.

It is recommended—

That the referee for 1908-9 be requested to study the following subjects as fully as may be practicable:

- (1) The official method for determining casein as indicated under 1.
- (2) The perfecting of the method for determining milk albumin.

## REPORT ON SUGAR.

By A. H. BRYAN, *Referee*, and FRITZ ZERBAN, *Associate Referee*.

The work of the referee and associate referee upon sugar during the past year has been substantially along the lines recommended by the association at its last meeting and has comprised (1) work upon special methods of analysis in their relationship to sugar chemistry; (2) work upon purely chemical methods; and (3) work by a number of collaborators upon methods for the analysis of cane molasses and sugars.

In the investigations of special methods the work has been confined very largely to the study of the application of the refractometer to the estimation of dry substance in the liquid sugar products. This study was published in the *Journal of the American Chemical Society*<sup>a</sup> and is not here repeated, but a recommendation based on the work is made.

The associate referee has confined his work mostly to the study of methods of estimating reducing sugars, trying the Monroe-Neubauer crucible (a platinum gooch with a filtering substance of platinum sponge), instead of the ordinary porcelain gooch. The results are given in the *Journal of the American Chemical Society*.<sup>b</sup>

The collaborative work consisted of two lines of determinations: (1) Methods of moisture determinations; (2) effect of clarifying agents on the polarization. Two samples were sent out, one of a yellow sugar and the other an open kettle cane molasses. In the circular letter sent out with these samples, the following instructions were given:

## INSTRUCTIONS.

- (1) *Moisture on both samples.*
  - (a) Two grams of material on sand to constant weight in vacuum oven at 70° C.
  - (b) Two grams of material without sand to constant weight in vacuum at 70° C.
  - (c) Two grams of sample on sand in water-jacketed oven for ten consecutive hours. Weigh at end of ten hours. Then heat for two-hour intervals until weight is constant.
  - (d) Repeat (c), but do not use sand.

<sup>a</sup>1908, 30: 1443.

<sup>b</sup>1908, 30: 1456.

(e) Two grams of sample on sand in water-jacketed oven for six hours on one day and four hours the following day. Weigh at end of the ten hours. Then heat for two-hour intervals until constant weight is attained.

(f) Same as (e), but do not use sand.

(g) By refractometer. The procedure is the same as for any work with the refractometer. The readings are taken at 28° C. or any other temperature. A few drops of the solution are placed on the prism and the border line adjusted and read as per instructions found in Bulletin 107, page 132. The per cent of water is obtained from table of Geerlig's herewith. A table of temperature corrections is also given, so that corrections can be made for any other temperature.

*Geerlig's table for dry substance in sugar-house products by the Abbe refractometer, at 28° C.<sup>a</sup>*

Index.	Per cent dry substance.	Decimals to be added for fractional readings. <sup>b</sup>		Index.	Per cent dry substance.	Decimals to be added for fractional readings. <sup>b</sup>	
1.3335	1	0.0001-0.05	0.0010-0.75	1.4083	45	0.0004=0.2	0.0015=0.75
1.3349	2	0.0002-0.1	0.0011-0.8	1.4104	46	0.0005=0.25	0.0016=0.8
1.3364	3	0.0003-0.2	0.0012-0.8	1.4124	47	0.0006=0.3	0.0017=0.85
1.3379	4	0.0004-0.25	0.0013-0.85	1.4145	48	0.0007=0.35	0.0018=0.9
1.3394	5	0.0005-0.3	0.0014-0.9	1.4166	49	0.0008=0.4	0.0019=0.95
1.3409	6	0.0006-0.4	0.0015-1.0	1.4186	50	0.0009=0.45	0.0020=1.0
1.3424	7	0.0007-0.5	.....	1.4207	51	0.0010=0.5	0.0021=1.0
1.3439	8	0.0008-0.6	.....	1.4228	52	0.0011=0.55	.....
1.3454	9	0.0009-0.7	.....	1.4249	53	.....	.....
1.3469	10	.....	.....	1.4270	54	.....	.....
1.3484	11	0.0001-0.05	.....	1.4292	55	0.0001=0.05	0.0013=0.55
1.3500	12	0.0002-0.1	.....	1.4314	56	0.0002=0.1	0.0014=0.6
1.3516	13	0.0003-0.2	.....	1.4337	57	0.0003=0.1	0.0015=0.65
1.3530	14	0.0004-0.25	.....	1.4359	58	0.0004=0.15	0.0016=0.7
1.3546	15	0.0005-0.3	.....	1.4382	59	0.0005=0.2	0.0017=0.75
1.3562	16	0.0006-0.4	.....	1.4405	60	0.0006=0.25	0.0018=0.8
1.3578	17	0.0007-0.45	.....	1.4428	61	0.0007=0.3	0.0019=0.85
1.3594	18	0.0008-0.5	.....	1.4451	62	0.0008=0.35	0.0020=0.9
1.3611	19	0.0009-0.6	.....	1.4474	63	0.0009=0.4	0.0021=0.9
1.3627	20	0.0010-0.65	.....	1.4497	64	0.0010=0.45	0.0022=0.95
1.3644	21	0.0011-0.7	.....	1.4520	65	0.0011=0.5	0.0023=1.0
1.3661	22	0.0012-0.75	.....	1.4543	66	0.0012=0.5	0.0024=1.0
1.3678	23	0.0013-0.8	.....	1.4567	67	.....	.....
1.3695	24	0.0014-0.85	.....	1.4591	68	.....	.....
1.3712	25	0.0015-0.9	.....	1.4615	69	.....	.....
1.3729	26	0.0016-0.95	.....	1.4639	70	.....	.....
1.3746	27	0.0001-0.05	0.0012-0.6	1.4663	71	.....	.....
1.3764	28	0.0002-0.1	0.0013-0.65	1.4687	72	.....	.....
1.3782	29	0.0003-0.15	0.0014-0.7	1.4711	73	0.0001=0.0	0.0015=0.55
1.3800	30	0.0004-0.2	0.0015-0.75	1.4736	74	0.0002=0.05	0.0016=0.6
1.3818	31	0.0005-0.25	0.0016-0.8	1.4761	75	0.0003=0.1	0.0017=0.65
1.3836	32	0.0006-0.3	0.0017-0.85	1.4786	76	0.0004=0.15	0.0018=0.65
1.3854	33	0.0007-0.35	0.0018-0.9	1.4811	77	0.0005=0.2	0.0019=0.7
1.3872	34	0.0008-0.4	0.0019-0.95	1.4836	78	0.0006=0.2	0.0020=0.75
1.3890	35	0.0009-0.45	0.0020-1.0	1.4862	79	0.0007=0.25	0.0021=0.8
1.3909	36	0.0010-0.5	0.0021=1.0	1.4888	80	0.0008=0.3	0.0022=0.8
1.3928	37	0.0011-0.55	.....	1.4914	81	0.0009=0.35	0.0023=0.85
1.3947	38	.....	.....	1.4940	82	0.0010=0.35	0.0024=0.9
1.3966	39	.....	.....	1.4966	83	0.0011=0.4	0.0025=0.9
1.3984	40	.....	.....	1.4992	84	0.0012=0.45	0.0026=0.95
1.4003	41	.....	.....	1.5019	85	0.0013=0.5	0.0027=1.0
1.4023	42	0.0001-0.05	0.0012=0.6	1.5046	86	0.0014=0.5	0.0028=1.0
1.4043	43	0.0002-0.1	0.0013=0.65	1.5073	87	.....	.....
1.4063	44	0.0003-0.15	0.0014=0.7	1.5100	88	.....	.....
				1.5127	89	.....	.....
				1.5155	90	.....	.....

<sup>a</sup> Intern Sugar J., 10: 69-70.

<sup>b</sup> Find in the table the refractive index which is next lower than the reading actually made and note the corresponding whole number for the per cent of dry substance. Subtract the refractive index obtained from the table from the observed reading; the decimal corresponding to this difference, as given in the column so marked, is added to the whole per cent of dry substance as first obtained.

Table of corrections for the temperature.

Dry substance.													
Temperature of the prisms in ° C.	0	5	10	15	20	25	30	40	50	60	70	80	90
Subtract—													
20.....	0.53	0.54	0.55	0.56	0.57	0.58	0.60	0.62	0.64	0.62	0.61	0.60	0.58
21.....	.46	.47	.48	.49	.50	.51	.52	.54	.56	.54	.53	.52	.50
22.....	.40	.41	.42	.42	.43	.44	.45	.47	.48	.47	.46	.45	.44
23.....	.33	.33	.34	.35	.36	.37	.38	.39	.40	.39	.38	.38	.38
24.....	.26	.26	.27	.28	.28	.29	.30	.31	.32	.31	.31	.30	.30
25.....	.20	.20	.21	.21	.22	.22	.23	.23	.24	.23	.23	.23	.22
26.....	.12	.12	.13	.14	.14	.15	.15	.16	.16	.16	.15	.15	.14
27.....	.07	.07	.07	.07	.07	.07	.08	.08	.08	.08	.08	.08	.07
Add—													
29.....	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.07
30.....	.12	.12	.13	.14	.14	.14	.15	.15	.16	.16	.16	.15	.14
31.....	.20	.20	.21	.21	.22	.22	.23	.23	.24	.23	.23	.23	.22
32.....	.26	.26	.27	.28	.28	.29	.30	.31	.32	.31	.31	.30	.30
33.....	.33	.33	.34	.35	.36	.37	.38	.39	.40	.39	.38	.38	.38
34.....	.40	.41	.42	.42	.43	.44	.45	.47	.48	.47	.46	.45	.44
35.....	.46	.47	.48	.49	.50	.51	.52	.54	.56	.54	.53	.52	.50

(h) By areometric methods, as found on pages 65–67, Bulletin 107. If time permits it would be well to determine the per cent of water in a number of sugar solutions by methods (g) and also (h) and (b). The comparison being between results of (g) and (b) and (h) and (b). The results could be reported as special samples under (g), giving kind of sirup, also figures obtained by (g), (b), and (h).

(2) *Polarimetric determinations.* *Effect of various clarifying agents on both samples.*

Weigh out a normal weight and make up to 100 cc, or to such a multiple thereof as may be necessary to secure an accurate polarization, after clarifying as follows:

(a) With lead subacetate solution. (Bull. 46, pp. 38–39; also Bull. 107, p. 40.) Try at least two different quantities of the clarifying agents, reporting the separate polarization.

(b) With normal lead acetate solution. (Saturated solution of lead acetate in water.)

(c) With Horne's dry lead subacetate. (J. Amer. Chem. Soc., 1904, 26:186.)

(d) With Herles' solution. No. 1, 250 grams lead nitrate to 500 cc water; No. 2, 25 grams sodium hydroxid to 500 cc water. Use equal parts of each solution, either 5 cc each or up to 10 cc of each. Note whether increased amount changes the polarization.

(e) With alumina cream and hydrosulphite (sodium hydrosulphite, B. A. S. F. or "Blankit"). This with the dry subacetate can be obtained from any of the large dealers in chemical supplies. In this clarification make solution up to required volume, then add a few crystals at a time until decolorization is effected. Polarize at once after filtering and again after standing for some time. Should the solution become cloudy on standing add some kaolin and shake, filter. Also try the following method of procedure: In a solution after clarifying with alumina cream and filtering, just before screwing on cap of polarization tube, add a few crystals of the hydrosulphite and shake. Polarize immediately; note whether on standing there is a change in the polarization.

(f) Invert portions of a, b, c, d, and e and determine the invert reading. Where lead has been used take out the excess with some crystals of potassium oxalate or dry sodium carbonate. Inversion can be accomplished by following (c), page 40, Bulletin 107, or (1), page 39, Bulletin 46, Revised. If the latter reference is used, the equation should read:

$$S = \frac{a-b}{142.66 - \frac{t}{2}}$$



In this polarization take care to record all temperatures of polarization, dilutions, etc., that results may be compared upon as uniform a basis as possible.

It is also urged that the work on the samples be begun immediately upon their arrival, to avoid changes in composition which might result from fermentation.

A. HUGH BRYAN,  
Referee on Sugar.  
FRITZ ZERBAN,  
Associate Referee.

A number of chemists signified their willingness to cooperate, and reports, in whole or in part, were received from them.

#### DETERMINATION OF TOTAL SOLIDS.

The work outlined was for the comparison of the vacuum method with the regular method of drying for ten hours. But as ten hours is generally longer than the ordinary laboratory day, a comparison was made of this determination conducted for ten consecutive hours, and also for six hours on one day and four the next. Together with the comparison of methods of determining moisture, the effect of mixing sand with the material to be dried was studied in each case. The refractometer was tried, and the specific gravity was also determined and the moisture calculated.

#### Determinations of moisture in sugar and molasses.

##### SUGAR.

Analyst.	Vacuum.		At boiling water temperature.				Refractometer.	Specific gravity.
			10 consecutive hours.		6 and 4 hours.			
	Sand.	No sand.	Sand.	No sand.	Sand.	No sand.		
P. H. Doherty, New Orleans, La.....	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
F. W. Liepsner, Washington, D. C....	a 2.84	a 2.57	Not dry.	2.68	2.87	2.76	2.60	2.22
W. P. Naquin, New Orleans, La.....			b 2.10	2.89	Not dry.	2.97	2.60	
John H. Parkins, Richmond, Va.....			2.68	2.79	2.41	2.37		
F. G. Smith, New Orleans, La.....			2.69	2.74	2.90	2.75	2.60	
R. N. Wilson, Florida.....	c 2.52	c 2.54	2.87	2.48	2.50	2.49		
Average.....	2.68	2.56	2.74	2.75	2.72	2.67	2.60	2.22

##### MOLASSES.

P. H. Doherty, New Orleans, La.....			b 28.06	27.67	27.99	27.37	27.04	26.36
F. W. Liepsner, Washington, D. C....	a 27.04	a 27.17	27.35	27.48	27.84	27.15	26.27	24.30
W. P. Naquin, New Orleans, La.....			27.38	b 25.55	27.94	27.86	27.04	26.36
J. H. Parkins, Richmond, Va.....			27.55	27.26	27.77	27.24	28.50	d 28.50
F. G. Smith, New Orleans, La.....			27.51	27.20	28.19	27.45	27.03	
Average.....	27.04	27.17	27.45	27.40	27.94	27.41	27.17	25.78

a Constant at end of 31 hours.  
b Not included in average.

c Constant at end of 10 hours.  
d By Westphal balance, 23.40.

It is noted here that the ten-hour drying gives higher results than the vacuum method. This is due, no doubt, to a decomposition of the material at the temperature of boiling water. The ten consecutive hour results are lower than when the time is divided. The use of sand plays an important part in the drying, the determinations being higher with sand present. The reason for this is self-evident. The material forms a coating on the sand and between the particles and so presents a larger surface to be affected by the heat. When not used, a hard, dry film forms on

the material and the under layer does not dry. When a small area of liquid is exposed for drying, the amount of moisture going off will be smaller than when a larger surface is exposed. Many chemists prefer and recommend the use of powdered pumice instead of sand. This allows the material to be absorbed. In the referee's opinion, the results so obtained are of no more value than those with the use of sand. Where numerous determinations are to be made, it is an easy matter to wash and clean the sand, while to clean the pumice stone and remove all traces of the sirup is not so easy. Lately the use of a roll of filter paper has been recommended <sup>a</sup> as the absorbent. Wiley (Bureau of Chemistry, Bul. 19, p. 49) recommended that in 1888, but it was thought then to give low results. Mintz by this method reduced the time of drying from seventeen hours to three. This method is practically the method of Adams for milk, and should be given some consideration for next year. Finely flaked asbestos as an absorbent material has been spoken of for drying milk. Browne <sup>b</sup> used it with success in determinations of moisture in apple juices. It is further worthy of trial, since the claim is made that it requires less time for drying than when sand or pumice stone is used. The referee has made a few experiments with the Soxhlet oven, where a current of dry air passes over the material, but the work has not progressed far enough to make a report. A method that bids fair to supersede all others for pure sugar solutions is the use of the refractometer. The comparison of this method made by the collaborators shows its results to be nearer the vacuum results than those of other methods. A second feature of the moisture work was a study of the effect of increasing the time of heating or dryness on the determination. The following table gives the average results obtained by the collaborators:

*Determinations of moisture increasing the time of drying.*

[Averages based on reports of five collaborators.]

SUGAR.

Modifications of method.	Pre-scribed time of heating.	By heating.						
		12 hours.	14 hours.	16 hours.	18 hours.	20 hours.	22 hours.	24 hours.
On sand:	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
Vacuum.....	2.68	.....	.....	.....	.....	.....	.....	.....
10 consecutive hours.....	2.58	2.78	2.91	2.89	3.04	3.06	.....	.....
6 and 4 hours.....	2.72	2.79	2.86	2.92	3.01	3.06	.....	.....
No sand:								
Vacuum.....	2.56	.....	.....	.....	.....	.....	.....	.....
10 consecutive hours.....	2.75	2.85	2.87	2.97	3.05	3.00	.....	.....
6 and 4 hours.....	2.67	2.70	2.81	2.84	2.97	2.97	.....	.....
Refractometer.....	2.45	.....	.....	.....	.....	.....	.....	.....

MOLASSES.

On sand:								
Vacuum.....	27.04	.....	.....	.....	.....	.....	.....	.....
10 consecutive hours.....	27.45	27.86	28.24	28.42	28.50	28.50	28.85	28.88
6 and 4 hours.....	27.94	28.19	28.53	28.67	28.82	28.78	29.02	.....
No sand:								
Vacuum.....	27.17	.....	.....	.....	.....	.....	.....	.....
10 consecutive hours.....	27.40	27.43	27.64	27.98	28.20	28.28	28.44	28.58
6 and 4 hours.....	27.41	27.68	27.85	28.15	28.31	28.47	28.58	.....
Refractometer.....	27.17	.....	.....	.....	.....	.....	.....	.....

From these figures the importance of not allowing the length of time to exceed ten hours is noted, as active decomposition sets in. This decomposition was greater when sand was used than when it was not, a result one would naturally expect.

<sup>a</sup> Centrbl. Zuckerind., 1908, 16 : 1102; Chemical Abstracts, 1908, 2 : 2632.

<sup>b</sup> J. Amer. Chem. Soc., 1901, 23 : 873.

## EFFECT OF CLARIFICATION AGENTS ON POLARIZATION.

The work carried on was a continuation of that taken up a number of years ago. The clarifying agents studied were neutral lead acetate, subacetate of lead, both wet and dry, Herles' solution or basic lead nitrate, and hydrosulphites. An additional feature was the comparison of the results obtained when using the necessary amount of the precipitant and when using an excess.

The results on the sugar and molasses samples will be considered separately and for a better comparison the results obtained by using the necessary amount of clarifying agent will be discussed. Following this the results of using an excess of clarifying agent will be considered.

*Polarizations of sugar with different clarifying agents, using only amount necessary for clarification.*

[Normal weight to 100 cc; polarized in 200 mm tube; sucrose factor 142.66.]

## SUBACETATE OF LEAD.

Analyst.	Amount of clarifying agent.	Direct polarization.	Corrected Invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
	cc.	°V.	°V.	°C.	Per cent.
A. W. Blair, Florida.....	5	93.00	-27.60	28	93.73
P. H. Doherty, New Orleans, La.....	2	93.00	-28.60	27	94.14
J. A. Hall, New York City.....	a 1	92.80	-29.86	25.5	94.43
G. H. Hardin, New York City.....	a 1	92.85	-29.20	27	94.50
W. D. Horne, Yonkers, N. Y.....	b 1	c 92.85	-----	22	c 92.85
W. L. Howells, New Orleans, La.....	d 1	93.40	-26.20	30	93.69
W. P. Naquin, New Orleans, La.....	2	93.22	-27.52	30	94.66
J. H. Parkins, Richmond, Va.....	d 5	c 90.00	-29.04	28	c 92.52
F. G. Smith, New Orleans, La.....	1	93.05	-27.02	27.5	93.14
M. H. Wiley, New York City.....	a 0.5	92.55	-30.03	25.5	94.38
R. N. Wilson, Florida.....	4	93.80	-27.60	27.4	94.14
F. Zerban, New Orleans, La.....	1	92.80	-30.36	26.0	94.99
Average.....		93.05	-----	-----	94.18

## DRY SUBACETATE OF LEAD.

	Grams.				
A. W. Blair, Florida.....	0.5	92.85	-27.40	28.0	93.46
P. H. Doherty, New Orleans, La.....	.5	92.60	-29.92	24.5	93.94
J. A. Hall, New York City.....	.5	92.70	-29.86	25.5	94.35
G. H. Hardin, New York City.....	.5	92.70	-29.15	27	94.34
W. D. Horne, Yonkers, N. Y.....	.27	c 92.70	-29.00	22	c 92.44
W. L. Howells, New Orleans, La.....	.5	93.20	-26.10	30	93.45
W. P. Naquin, New Orleans, La.....	.1	92.96	-28.40	29	94.67
J. H. Parkins, Richmond, Va.....		93.40	-28.60	28	94.82
F. G. Smith, New Orleans, La.....		92.89	-27.07	27.5	93.09
M. H. Wiley, New York City.....	.5	92.50	-29.92	25.5	94.23
R. N. Wilson, Florida.....	.5	93.41	-26.49	28.8	93.48
F. Zerban, New Orleans, La.....	1.0	92.80	-29.15	26.5	94.24
Average.....		92.90	-----	-----	94.00

## NEUTRAL LEAD ACETATE.

	cc.				
A. W. Blair, Florida.....	2	92.90	-27.40	28	93.58
P. H. Doherty, New Orleans, La.....	3	92.80	-28.60	27	93.98
J. A. Hall, New York City.....	2	92.60	-29.97	25.5	94.28
G. H. Hardin, New York City.....	5	92.80	-29.75	26.0	94.52
W. D. Horne, Yonkers, N. Y.....	e 3.6	92.40	-----	22.0	-----
W. L. Howells, New Orleans, La.....	1	93.20	-26.50	30	93.77
W. P. Naquin, New Orleans, La.....	3	93.10	-27.85	29.3	94.48
J. H. Parkins, Richmond, Va.....	5	c 92.00	-29.04	28.0	c 94.07
F. G. Smith, New Orleans, La.....	f 1	93.14	-27.30	27.5	93.43
M. H. Wiley, New York City.....	2	92.75	-30.03	25.5	94.38
R. N. Wilson, Florida.....	3	93.58	-27.03	28.8	94.04
F. Zerban, New Orleans, La.....	1	92.90	-28.82	26.5	93.98
Average.....		92.92	-----	-----	94.04

a 54.3 brix.  
b 56.0 brix.

c Not included in average.  
d 1.25 sp. gr.

e 10 per cent solution.  
f 20 per cent solution.

*Polarizations of sugar with different clarifying agents, using only amount necessary for clarification—Continued.*

BASIC LEAD NITRATE (HERLES' SOLUTION).

Analyst.	Amount of clarifying agent.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
	cc each.	°P <sub>100</sub>	°V.	°C.	Per cent.
P. H. Doherty, New Orleans, La. ....	2	92.60	-29.70	24.5	93.78
J. A. Hall, New York City. ....	2	92.75	-29.86	25.5	94.40
G. H. Hardin, New York City. ....	5	92.80	-29.86	26.0	94.60
W. D. Horne, Yonkers, N. Y. ....	0.72	92.80			
W. L. Howells, New Orleans, La. ....	5	93.50	-25.50	30.0	93.21
W. P. Naquin, New Orleans, La. ....	5	93.32	-28.82	28.6	95.16
J. H. Parkins, Richmond, Va. ....	5	<sup>a</sup> 92.22	-29.70	28.0	<sup>a</sup> 94.76
F. G. Smith, New Orleans, La. ....	5	93.44		28.5	
M. H. Wiley, New York City. ....	5	92.80	-30.14	25.5	94.57
F. Zerban, New Orleans, La. ....	1	92.80	-30.47	26.0	95.07
Average. ....		92.98			94.39

ALUMINA CREAM AND SODIUM HYDROSULPHITE.

P. H. Doherty, New Orleans, La. ....		92.20			
W. D. Horne, Yonkers, N. Y. ....	0.7	92.60			
W. L. Howells, New Orleans, La. ....		93.10	-25.80	30.0	93.14
J. H. Parkins, Richmond, Va. ....		<sup>a</sup> 92.00	-30.80	28.0	<sup>a</sup> 95.44
F. G. Smith, New Orleans, La. ....		93.25	-26.90	29.0	93.75
R. N. Wilson, Florida. ....		<sup>a</sup> 94.20	-26.83	28.8	<sup>a</sup> 94.37
F. Zerban, New Orleans, La. ....		92.50	-31.24	26.0	95.43
Average. ....		92.75			94.11

<sup>a</sup> Not included in average.

The results of individual analysts on direct polarization compare very favorably in each method of clarification. There are, however, some higher figures than the average, but in every case the polarization was carried on at a lower temperature or an excess of the precipitant was used. With a few exceptions the results on sucrose by the Clerget method do not differ so widely as the direct polarizations. This difference with the Clerget method is most likely due to the different methods of inverting or to an error in calculation. As a check for the first error it is well to run a test on pure sucrose with each set of determinations. This is especially wise in case of inverting by heat, as the temperature may not be right or the time either too short or too long, and, as a result, either all the sucrose has not been inverted, or the inversion has been carried on so far that reversion products have been formed. Even in the standing method for inversion this blank is valuable in determining the completeness of the inversion. There is a point in the cold inversion that should receive some attention. This is the question of the relation of time and temperature. In a few experiments on the same sugar solution, one inverted by standing at 20° C. for twenty hours showed -12.3, while the other portion by standing at 32° for twenty hours showed -12.08. These figures would make a difference in the Clerget sucrose.

The other point, error in calculation, is one that for some reason or other is rather common. The inversion is carried on by taking 50 cc of the solution and adding 5 cc of acid and not correcting the reading for the increase of 10 per cent in volume. Chemists not using the formula often should guard against this error, as the difference amounts to nearly 3 per cent in high polarizations.

In comparing the average results of direct polarization it is noted that hydrosulphite gives the lowest reading, while wet subacetate gives the highest. The normal acetate and Herles' solution give nearly the same results. Dry subacetate gives readings that are lower than the two above mentioned and stands next to hydrosulphite.

As regards the decolorization effect, Herles' solution equals wet subacetate. The dry subacetate gives solutions a little darker than the above, and next in order is neutral acetate. Hydrosulphite gives a good decolorization, but under certain conditions the solutions become murky from the precipitation of sulphur and also, on standing, the color returns again.

When the precipitation agents are used in excess, the readings are all higher, as shown by the following table. This increase is no doubt due largely to the solution becoming more concentrated from the increased amount of precipitation, and partly also from a change in rotation due to the salts.

*Polarizations of sugar with different clarifying agents, using an excess of clarifier.*

[Normal weight to 100 cc; polarized in 200 mm tube; sucrose factor 142.66.]

SUBACETATE OF LEAD.

Analyst.	Amount of clarifying agent.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
	cc.	° Y.	° Y.	° C.	Per cent.
P. H. Doherty, New Orleans, La.	3	93.00	-28.60	27	94.14
J. A. Hall, New York City	a 4	92.95	-29.70	25.5	94.42
G. H. Hardin, New York City	a 3	92.95	-29.20	27	94.58
W. D. Horne, Yonkers, N. Y.	b 2	92.90		22	
W. L. Howells, New Orleans, La.	c 2	93.50	-26.00	30	93.60
W. P. Naquin, New Orleans, La.	3	93.24	-27.56	30.3	94.74
J. H. Parkins, Richmond, Va.	c 10	d 91.60	-29.04	28.0	d 93.77
F. G. Smith, New Orleans, La.	2	93.15	-27.30	27.5	93.44
M. H. Wiley, New York City	a 3	92.80	-29.70	25.5	94.30
R. N. Wilson, Florida	6	94.00	-27.00	28.0	94.05
F. Zerban, New Orleans, La.	2	92.80	-30.25	25.5	94.72
	3	92.90	-30.14	25.5	94.71
Average		93.11			94.26

DRY LEAD SUBACETATE.

	Grams				
P. H. Doherty, New Orleans, La.	1.00	92.60	-29.70	24.5	93.75
W. D. Horne, Yonkers, N. Y.	.75	92.75			
W. P. Naquin, New Orleans, La.	.50	93.26	-28.53	28.8	94.95
R. N. Wilson, Florida	1.00	93.61	-26.63	29.0	93.82
F. Zerban, New Orleans, La.	2.00	92.50	-29.15	26.5	94.00
Average		92.94			94.13

NEUTRAL LEAD ACETATE.

	cc.				
P. H. Doherty, New Orleans, La.	4	92.80	-28.60	27.5	94.18
W. P. Naquin, New Orleans, La.	5	93.28	-27.98	29.2	94.69
J. H. Parkins, Richmond, Va.	10	d 91.00	-29.04	28.0	d 93.30
F. Zerban, New Orleans, La.	2	92.80	-29.26	26.5	94.32
Average		92.96			94.39

BASIC LEAD NITRATE.

	cc each.				
P. H. Doherty, New Orleans, La.	4	92.80	-29.70	25.0	94.11
W. L. Howells, New Orleans, La.	10	93.60	-25.50	30.0	93.29
W. P. Naquin, New Orleans, La.	10	93.20	-33.22	18.2	94.65
F. G. Smith, New Orleans, La.	10	93.55		28.5	
F. Zerban, New Orleans, La.	2	93.00	-30.47	26.0	95.22
Average		93.23			94.32

a 54.3 brix.

b 56 brix.

c 1.25 sp. gr.

d Not included in average.

The greatest difference is in the Herles' solution, then comes the wet and dry subacetate, which show about the same increase, and the least increase is with normal

acetate. This would naturally be expected, as the Herles' solution forms a precipitate in itself, hence causing concentration, and the excess of wet lead subacetate causes an increase and also a change of precipitate, thereby changing the concentration, while the normal acetate produces no more precipitate with an excess, and hence no change of concentration.

As regards the danger of adding an excess, this is the least in case of the neutral acetate, as an excess is indicated when no more precipitate continues to form. When using wet subacetate a better clarification is reached before the point where more acetate will produce a further precipitation. With dry lead it is difficult to determine when enough has been added. To add by weight takes much time, but where many determinations are to be made varying measures or cups could be used, the weight of the contents having been previously determined. It has the fault of precipitating reducing sugars in as large quantities as the wet subacetate, as noted in last year's report; besides, an excess of this reagent increases the volume of the solution, thereby lowering polarization. This effect is shown in the following experiment: Six hundred cubic centimeters of a solution of pure sucrose were made up and five 100 cc flasks were filled and the following quantities of the dry subacetate were added, shaken, and then polarized, care being taken that the polarization was made at 20° C.

*Polarization of pure sucrose solution with varying amounts of dry lead.*

Number.	Dry subacetate.	Polarization.
	<i>Grams.</i>	<i>° V.</i>
1.....	0.0	81.25
2.....	.5	81.1
3.....	1.0	81.05
4.....	1.5	81.0
5.....	2.0	80.9

It is noted from the table that the polarization has been lowered 0.35° by the addition of 2 grams of the dry lead.

The greater part of the lead subacetate went into solution even up to the 2 gram quantity, and only a cloud was noted. The meniscus of the liquid in the flasks containing the added lead subacetate was above the 100 mark in each case, showing an increase in volume.

Experiments were tried to determine this increase in volume. Five accurately graduated flasks with glass stoppers were used, and into these were weighed the varying quantities of dry subacetate, as in the previous experiment. A 100 cc pipette was used and an equal amount of solution of sucrose was added to each flask, the flasks being shaken during the addition of the solution. When added, the flasks were corked up and allowed to stand over night. The height of the liquid being marked on the neck of the flasks, the contents were poured out and the flasks cleaned and dried. By means of a Morse-Blalock pipette, capable of reading to 0.005 cc, the flasks were filled to the mark. The results are tabulated below:

*Volumes of solution of pure sucrose when adding various amounts of dry subacetate.*

No.	Dry subacetate of lead added.	Content.	Calculated to 100.
	<i>Grams.</i>	<i>cc.</i>	<i>cc.</i>
1	0.0	99.90	100.00
2	.5	99.91	100.01
3	1.0	100.02	100.12
4	1.5	100.18	100.28
5	2.0	100.21	100.32

An increase of 0.32 cc in volume by the addition of 2 grams of dry subacetate is noted, and, with a solution polarizing  $81.25^\circ$ , as in the experiment given above, the calculated polarization for this increase in volume would be  $80.99^\circ$ . The solution actually polarized  $80.9^\circ$ . Horne <sup>a</sup> gives 0.22 cc as the increase in volume on 1 gram of subacetate. Pellet has shown it to be 0.37 cc. The referee's sugar sample for this year, worked as above described, showed the following changes in volume in two experiments:

*Changes in volume using official sugar samples.*

No.	Dry sub-acetate lead added.	Experiment 1.	Experiment 2.
	<i>Grams.</i>		
1	0.0	100.00	100.00
2	.5	100.09	100.14
3	1.0	100.25	100.19
4	1.5	100.32	100.34
5	2.0	100.53	100.58

An average increase in volume of about 0.55 cc is noted, this being due to the precipitate formed and also to the fact, as shown above, of the solution of the lead subacetate.

From these experiments it is seen that clarification with dry lead introduces the same errors as with wet lead, viz., a precipitation of the reducing sugars, and also where used to excess a change in volume. The latter effect with wet lead acetate as a clarifier tends to raise the readings, while with dry lead there is a tendency to lower them. However, in using the dry subacetate of lead the errors are compensating, since the increase in volume tends to lower the reading and the precipitation of the levulose to raise it, while with wet subacetate the volume is decreased by the formation of the precipitate, hence the reading increased, and this is again increased by the precipitation of the levulose. Dry lead subacetate is a step in advance in the search for the best clarifying agent, and further experiments are in progress; but so far the perfect clarifying agent for dark-colored sugar solutions has not been found.

As to the use of hydrosulphites as a bleach for solutions to be polarized there are serious objections. When large quantities of reducing sugars are present in the sample the reading is lowered. This was pointed out at last year's meeting by the writer. The rotation of one of the sugars, dextrose, is decidedly lowered; hence the polarization is lowered if the sample contains much dextrose. This change of rotation of dextrose is due to the formation of an oxysulphonate which has a levotation. The dissociation of the glucose (dextrose) oxysulphonate can be measured by this fact. In the experiments cited no inversion of sucrose by this substance was noted, but later literature shows numerous cases of inversion by using commercial hydrosulphite.

Where the quantity of reducing sugars is small, there is very little reduction in the polarization due to the formation of this compound, and it has this merit, that readings are not vitiated by a change in volume due to a precipitate. These compounds, hydrosulphites, while stable under most conditions, are very easily decomposed in moist air and also on long standing, and hence lose their power of decolorization. And again, their power of decolorization is limited, as they have no effect on caramel bodies (those which give the dark color to molasses) but do bleach intermediate substances, which on longer heating would yield caramel.

<sup>a</sup> J. Amer. Chem. Soc., 1907, 29: 928.

## MOLASSES SAMPLE.

Unfortunately the sample of molasses selected for the collaboration work was of such a consistency that fermentation started after shipping, and the results are not of such value as they might have been had this not occurred. The reserve sample also was found to be fermented, so that it was not possible to make check results.

The results as received from the collaborators are given here in tabular form.

*Polarization of molasses with different clarifying agents, using only amount necessary for clarification.*

## SUBACETATE OF LEAD.

Analyst.	Amount of clarifying agent.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
	cc.	° V.	° V.	° C.	Per cent.
A. W. Blair, Florida.....	5	42.56	-20.00	22.0	47.51
P. H. Doherty, New Orleans, La.....	8	43.00	-18.26	26.5	47.33
W. L. Howells, New Orleans, La.....	6	<sup>a</sup> 43.40	-15.80	23.2	<sup>a</sup> 45.17
W. P. Naquin, New Orleans, La.....	8	42.42	-20.59	22.6	47.90
J. H. Parkins, Richmond, Va.....	5	42.40	-17.60	26.3	46.33
F. G. Smith, New Orleans, La.....	6	43.72	-17.35	27.5	47.38
F. Zerban, New Orleans, La.....	5	<sup>a</sup> 41.80	-19.36	27.0	<sup>a</sup> 47.35
Average.....		42.82			47.29

## DRY SUBACETATE OF LEAD.

Analyst.	Grams.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
		° V.	° V.	° C.	Per cent.
A. W. Blair, Florida.....	1.0	42.00	-19.04	22.0	46.37
P. H. Doherty, New Orleans, La.....	2.0	42.54	-17.82	27.5	46.86
W. L. Howells, New Orleans, La.....		<sup>a</sup> 43.10	-16.60	23.2	<sup>a</sup> 45.56
W. P. Naquin, New Orleans, La.....	2.0	42.36	-20.06	23.5	47.68
J. H. Parkins, Richmond, Va.....		<sup>a</sup> 42.60	-19.80	26.5	<sup>a</sup> 48.26
F. G. Smith, New Orleans, La.....		44.22	-16.85	27.5	47.37
F. Zerban, New Orleans, La.....	2.0	42.00	-20.02	26.0	47.82
Average.....		42.63			47.22

## NEUTRAL LEAD ACETATE.

Analyst.	cc.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
		° V.	° V.	° C.	Per cent.
A. W. Blair, Florida.....	2	42.00	-19.84	22.0	46.97
P. H. Doherty, New Orleans, La.....	12	42.80	-17.91	27.5	47.09
W. L. Howells, New Orleans, La.....	6	<sup>a</sup> 42.90	-14.00	23.0	<sup>a</sup> 43.42
W. P. Naquin, New Orleans, La.....	10	42.04	-20.10	23.4	47.45
J. H. Parkins, Richmond, Va.....	5	<sup>a</sup> 42.00	-22.00	26.3	<sup>a</sup> 49.49
F. G. Smith, New Orleans, La.....	6	43.47	-17.45	27.5	47.26
F. Zerban, New Orleans, La.....	5	42.00	-19.58	25.0	47.31
Average.....		42.46			47.22

## BASIC LEAD NITRATE.

Analyst.	cc each.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
		° V.	° V.	° C.	Per cent.
P. H. Doherty, New Orleans, La.....	5	42.60	-18.26	27.5	47.22
W. L. Howells, New Orleans, La.....	5	44.00	-17.40	23.2	46.86
W. P. Naquin, New Orleans, La.....	5	42.46	-20.24	23.5	47.89
J. H. Parkins, Richmond, Va.....	5	43.20	-19.80	26.5	48.68
F. G. Smith, New Orleans, La.....	5	43.90	-17.50	27.5	47.62
F. Zerban, New Orleans, La.....	5	<sup>a</sup> 41.90	-20.24	24.0	<sup>a</sup> 47.56
Average.....		43.23			47.65

## ALUMINA CREAM AND SODIUM HYDROSULPHITE.

Analyst.	Amount of clarifying agent.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
		° V.	° V.	° C.	Per cent.
P. H. Doherty, New Orleans, La.....		42.00			
W. L. Howells, New Orleans, La.....		42.40	-18.20	23.2	46.26
W. P. Naquin, New Orleans, La.....		41.96	-21.31	18.6	47.45
J. H. Parkins, Richmond, Va.....		42.00	-18.48	26.5	46.73
F. G. Smith, New Orleans, La.....		42.97	-17.53	29.0	47.21
F. Zerban, New Orleans, La.....		40.60	-21.34	24.0	47.41
Average.....		41.99			47.01

<sup>a</sup> Omitted from average.



A comparison of the average direct polarizations develops the fact that the hydrosulphites, as in the case of the sugar sample, give the lowest readings, neutral lead acetate next, and then dry and wet lead subacetate, which are about the same. The polarization with Herles' solution is the highest. The low polarization, when using hydrosulphites, has been already explained. Leaving that one out and the Herles' polarization, the other three agree fairly well. The calculations for sucrose by the Clerget formula give results that agree very closely. The highest is the Herles' result. When this reagent is used, the factor is not 142.66, but 143.5, due to the fact of the presence of a nitrate, instead of an acetate salt. Using this factor, the results would be lower.

*Polarization of molasses with different clarifying agents, using an excess of clarifier.*

SUBACETATE OF LEAD.

Analyst.	Amount of clarifying agent.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
		° V.	° V.	° C.	Per cent.
	cc.				
P. H. Doherty, New Orleans, La.....	10	43.24	-18.04	27.0	47.44
W. L. Howells, New Orleans, La.....	8	<sup>a</sup> 43.50	-15.12	23.2	<sup>a</sup> 44.73
W. P. Naquin, New Orleans, La.....	10	42.50	-19.75	24.2	47.68
J. H. Parkins, Richmond, Va.....	10	43.60	-17.60	26.3	46.55
F. G. Smith, New Orleans, La.....	8	43.92	-17.33	27.5	47.52
F. Zerban, New Orleans, La.....	7.5	42.00	-19.58	27.0	47.68
	10	42.30	-18.81	26.0	47.13
Average.....		42.94			47.33

DRY SUBACETATE OF LEAD.

	Grams.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
P. H. Doherty, New Orleans, La.....	3.0	43.04	-17.60	27.5	47.04
W. P. Naquin, New Orleans, La.....	3.0	42.85	-19.05	23.0	47.19
F. Zerban, New Orleans, La.....	4.0	42.40	-19.36	26.0	47.63
Average.....		42.76			47.29

NEUTRAL LEAD ACETATE.

	cc.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
P. H. Doherty, New Orleans, La.....	15	43.00	-18.26	27.5	47.52
W. P. Naquin, New Orleans, La.....	15	42.16	-20.28	23.0	47.60
J. H. Parkins, Richmond, Va.....	10	<sup>a</sup> 42.00	-22.00	26.3	<sup>a</sup> 49.49
F. Zerban, New Orleans, La.....	7.5	42.10	-19.58	25.0	47.39
	10.0	42.10	-19.58	25.0	47.39
Average.....		42.34			47.48

BASIC LEAD NITRATE.

	cc each.	Direct polarization.	Corrected invert polarization.	Temperature of polarization.	Sucrose by Clerget method.
P. H. Doherty, New Orleans, La.....	10	43.40	-17.82	27.0	47.39
W. P. Naquin, New Orleans, La.....	10	<sup>a</sup> 44.02	-20.15	23.5	<sup>a</sup> 49.02
W. L. Howells, New Orleans, La.....	10	<sup>a</sup> 44.40	-15.40	23.2	<sup>a</sup> 45.64
F. G. Smith, New Orleans, La.....	10	44.30	-17.47	27.5	47.92
F. Zerban, New Orleans, La.....	7.5	42.50	-19.03	26.5	47.55
	10.0	42.80	-18.70	27.0	47.62
Average.....		43.25			47.62

<sup>a</sup> Omitted from average.

When an excess of reagent is used all the polarizations are raised, as shown in the preceding tables. In the direct polarization, clarification with an excess of dry subacetate gives the least increase in polarization, while the greatest is noted with Herles' solution. Neutral acetate shows a lower reading when used in excess. The agreement in the Clerget calculation here is closer than in the other cases.

Summing up the work, it can be said that where reducing sugar determinations follow polarizations the clarifying agent should be neutral lead acetate. But for ordinary polarization work, where the reducing sugar content is not high, either sub-acetate or neutral acetate can be used. Where the content of invert sugar is high, a double polarization is necessary to obtain the correct figure for sucrose, and then any of the clarifying agents can be used, but care should be taken not to use an excess.

There is one point to which special attention should be called, namely, the estimation of reducing sugars. In Bulletin 107, Revised, page 53, under 6, Direct Weighing of Cuprous Oxid, the weighing as cuprous oxid will give too high results if the material under examination is high in nitrogenous matter or mineral salts; xanthin bases and some other nitrogenous bodies are thrown down by the Fehling solution along with the cuprous oxid. Also some salts are precipitated, and would be weighed as cuprous oxid, thereby giving false results. This has been conclusively shown by C. A. Browne in his reports as referee on sugar for the past two years, and is borne out in all of the referee's work. In such cases the copper of the precipitate must be determined direct either as cupric oxid or, better, by some volumetric method, as Low's, where the cuprous oxid is dissolved, treated, and finally the copper estimated by titration with thiosulphate. This is a longer procedure than the weighing as red oxid, but it should be done if reliable and accurate figures are to be obtained.

#### RECOMMENDATIONS ON SUGAR.

It is recommended—

(1) That the question of the influence of precipitants upon the polarization of sugars be further investigated.

(2) That the question of methods of determining moisture or dry substance be further investigated.

(3) That the method of determining dry substance by means of the refractometer and the table of Geerligs be adopted provisionally by the association.

(4) That under "Methods for the Determination of Copper contained in the Precipitate of Cuprous Oxid," pages 51-53, Official Methods, Bulletin 107, Revised (6) "Direct Weighing of Cuprous Oxid," there be a limitation inserted, viz: "This method should not be used in determining reducing sugars in commercial products, as other substances are precipitated along with the cuprous oxid. In these products the copper of the cuprous oxid should be determined direct by titration as in Low's method (*ibid.*, 241) or as cupric oxid."

#### DETECTION OF SMALL PERCENTAGES OF COMMERCIAL GLUCOSE IN SIRUPS AND HONEY.

By A. H. BRYAN, *Referee.*

The provisional method of this association is the one described on page 70 of Bulletin 107, Revised. It calls for a polarization of the inverted solution at 87° C. A dextrorotary reading at this temperature is said to be due to commercial glucose. And to obtain the percentage of glucose, the method divides this reading by the factor 163 and multiplies by 100.

C. A. Browne, in his report on honey, Bulletin 110, shows that normal honey naturally has a dextrorotation at 87° C. and hence the results of a determination by this method would not express the truth. The dextrorotation of a honey is due to honey dextrins. These are of a different character from those obtained by acid hydrolysis of starch, or such as occur in commercial glucose. One point of difference is the fact that honey dextrin is not colored by iodine solution, while the dextrins of glucose, except in cases of a high conversion product, are colored by iodine. By means of this test, Beck-

man's test,<sup>a</sup> as it is called, one can distinguish between these dextrins, and hence can say whether commercial glucose has been added. Browne called attention to the importance of this test. He also gave methods for the determination of the percentage of glucose present in mixtures. In the following table are given analyses of mixtures of different amounts of glucose and honey:

*Analyses of mixtures of commercial glucose and clover honey.*

Mixture.		Invert polarization—			Polarization difference (87° - 20°).	Invert sugar—		Calculated glucose.		
Glucose.	Honey.	Constant direct polarization at 20° C.	At 20° C.	At 87° C.		Before inversion.	After inversion.	Invert polarization at 87° + 1.63.	Invert polarization at (20° C. - 17.5) + 1.93.	100 - (corrected polarization difference) × 100 ÷ (26.7).
<i>Perct.</i>	<i>Perct.</i>	<i>° V.</i>	<i>° V.</i>	<i>° V.</i>	<i>° V.</i>	<i>Perc.</i>	<i>Perc.</i>	<i>Perc.</i>	<i>Perc.</i>	<i>Perct.</i>
100	.....	+153.8	+153.34	+144.32	.....	30.02	30.45	88.5	88.5	.....
50	50	+ 67.0	+ 65.67	+ 73.81	8.14	53.67	54.50	45.3	43.1	56.9
20	80	+ 15.4	+ 13.42	+ 33.00	19.58	69.00	70.35	20.2	16.0	19.2
10	90	- 2.4	- 4.84	+ 18.59	23.43	74.42	74.12	11.4	6.6	8.8
5	95	- 11.5	- 14.30	+ 11.66	25.96	75.74	77.80	7.2	1.6	3.8
3	97	- 14.2	- 16.94	+ 9.13	26.07	76.62	78.01	5.6	.29	3.7
2	98	- 16.0	- 18.70	+ 8.14	26.84	76.64	78.34	5.0	.00	1.2
1	99	- 18.2	- 20.90	+ 6.93	27.83	77.20	78.87	4.2	.00	.0
.....	100	- 19.5	- 22.11	+ 5.94	28.05	77.68	78.93	3.2	.00	.0

In the direct and invert polarizations it is noted that there is a gradual change from a "plus" polarization to a "minus" polarization, due to the increase of the glucose percentages. With reducing sugars before and after inversion there is again a large increase with the decrease of glucose. The difference in polarization of the inverted solution at 20° and 87°, as shown in column 4, increases from 8.14 to 28.05. C. A. Browne found that nearly 95 per cent of his samples of pure honey showed a difference ranging from 23 to 30 and the lowest was about 20. Taking 23 as a low figure, a mixture of 10 per cent of glucose with honey would not be considered adulterated. If, however, the natural honey had not shown such a high difference, viz, 28.05, then 10 per cent would be easily detected by this figure; but by adding up to 5 per cent this difference is not noticeable, and also the other analytical figures would not indicate the presence of glucose. It is, however, easily distinguishable when Beckman's test is applied to the honey. In fact, with the addition of as low as 1 per cent of glucose, its presence can be recognized by this test, especially if the dextrins are precipitated by alcohol and then dissolved in water, thereby concentrating them.

In the last three columns of the table the results of determining per cents of glucose by the three different methods are given. It is seen that the method proposed by Browne gives the figures closest to the actual mixture. Obviously in honey work Beckman's test should be employed in all cases, and in the hands of ordinary chemists after a few trials it will give good results.

As to the need for such a test, it is a well-known fact that where commercial invert sugar is used in a mixture of honey, also where honeys that crystallize are used, a small percentage of glucose is quite often added to prevent this crystallization. Cases are on record of such mixtures where less than 1 per cent of glucose was added. The iodine test will indicate the presence of glucose down to that amount.

Along this line the same question comes up in the examination of sirup and molasses. As is well known, glucose is added to these products in large quantities, and again in

other cases it is added in smaller quantities for the same purpose as in the case of honey. To be able to determine the small quantity is the problem.

In the first place, molasses or sirup from cane may show some polarization at 87° C. on the inverted solution. This polarization is generally to the right, though there are cases where it was to the left. This dextrorotation may be due to a preponderance of dextrose in the reducing sugars of the sample due to the easy decomposition of the levulose or may be due to the decomposition products formed when the raw juice is being defecated with lime, or by chance it might come from a special fermentation of the sample forming dextran. However, it can be said not to be due to dextrans. The normal polarization of sirups and molasses has been studied in samples of known purity from Louisiana and is given in the following table:

*Polarization of Louisiana molasses and sirup.*

MOLASSES.

Direct polarization at 20° C.	Corrected invert polarization—		Dry sub- stance.
	At 20° C.	At 87° C.	
° I.	° I.	° I.	<i>Per cent.</i>
40.8	-20.24	+2.2	80.8
24.6	-20.9	+2.2	76.8
26.0	-18.26	+3.52	76.8
42.4	-16.94	+2.42	78.2
52.4	-16.28	+2.20	69.1
55.6	-13.59	+4.18	69.6
39.6	-18.04	+2.20	80.8
39.6	-17.82	+2.20	79.0
44.0	-17.16	+2.64	72.0
42.0	-17.60	+2.42	73.8
42.4	-17.27	+3.52	76.1
41.6	-16.94	+3.96	74.0
52.4	-17.60	+3.52	76.1
26.6	-19.8	.00	78.1
50.8	-25.08	+1.10	87.5
22.6	-16.72	+3.96	84.1
41.6	-14.74	+1.10	75.0
45.6	-15.4	+2.20	78.0

SIRUP.

48.4	-17.6	+1.98	74.3
54.0	-18.7	+3.30	68.3
50.2	-12.1	+6.16	-----
50.4	-14.3	+1.76	-----
61.8	-16.5	+2.20	-----
Average.....		+2.65	-----
Maximum.....		+6.16	-----
Minimum.....		0.00	-----

It is noted that the average is +2.65, the minimum 0.00, and maximum +6.16. The sample giving +6.16 was badly fermented, hence its high figure. There remain about 100 samples to be examined, and when these are finished more definite figures can be obtained. As far as the work has progressed, about +5.5 is the maximum for the invert reading at 87° C. The iodine method spoken of in the honey work can be used here, but the sample must receive some previous treatment before applying the test. Ten grams of the sample can be diluted with a little water (if the sample be a molasses), and shaken with 95 per cent alcohol, adding a little at a time with shaking. The precipitate settles on standing. When settled, pour off the alcohol, add a little water to dissolve the precipitate, heating if necessary, and then reprecipitate with 95 per cent alcohol. Repeat a number of times. If the solution of this material in water is still dark in color, filter through charcoal, or, better, add a drop of hydrochloric acid and

reprecipitate the dextrans with alcohol. Wash the precipitate with 95 per cent alcohol, finally dissolve in water, and then test with iodine. A blank of pure water should also be treated with the same quantity of iodine solution and run with the test. A positive test of erythro-dextrin or amylo-dextrin is sufficient proof of the presence of commercial glucose.

Mr. Davidson, as chairman of the committee to present the question of the unification of terms to the International Congress of Applied Chemistry, stated that the committee would present the question according to their instructions at the meeting to be held May 29, 1909, and report the results to the association at its subsequent annual meeting.

## REPORT OF COMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By R. J. DAVIDSON, *Chairman.*

### NITROGEN.

Two recommendations (Nos. 2 and 4, Circular 38, p. 1) made by the referee in 1907, and referred to the referee for 1908, in regard to the use of copper sulphate in the Kjeldahl and Gunning methods, were again recommended for final action and were adopted. These changes in the official methods read as follows:

(1) Bulletin 107, revised, page 6, line 4, under "(3) Determination," after the words "sulphuric acid," insert: "From 0.1 to 0.3 gram of crystallized copper sulphate may also be used in addition to the mercury or in lieu of it."

(2) Bulletin 107, revised, page 7, line 4, under "(3) Determination," after the words "sulphuric acid," insert: "From 0.1 to 0.3 gram of crystallized copper sulphate may also be added."

Recommendations (3) and (4) offered by the referee for adoption were modified as follows and proposed by the committee for further work, which latter recommendation was adopted:

(3) Bulletin 107, revised, page 8, fourth line, after the word "time" insert: "Allow the flask to stand without heat for not less than six hours or for a shorter time with shaking at regular intervals."

(4) Bulletin 107, revised, page 8, under "(3) Determination," fifth line, after the word "and," insert the same sentence as in recommendation (3). The sentence then reads: "Add 5 grams of thiosulphate and allow the flask to stand without heat for not less than six hours or for a shorter time with shaking at regular intervals; then heat the solution for five minutes," etc.

### POTASH.

It is recommended—

(1) That the cobalti-nitrite method for potash be tested during the coming year. (See p. 124.)

Adopted.

(2) That there be a further trial of the method involving the use of ammonium hydroxid and ammonium oxalate in the preparation of the solution in the determination of the potash in potash salts, as compared with the present method of direct precipitation of the potash without the use of the reagents mentioned.

Adopted.

The referee stated that he had not been able to take up the extensive investigations that would be necessary in attempting to define available potash, and offered the following resolution, which was adopted by the association:

*Resolved,* That in view of the fact that practically the entire available time of the referee on potash is needed for the study of analytical methods, the investigation of

the question of determining what should be designated as "available potash," provided for in a resolution adopted in 1906, be undertaken by a special referee or associate referee.

#### PHOSPHORIC ACID.

It is recommended—

(1) That the recommendation of 1907 be repeated, namely, that the referee on phosphoric acid shall take up for report, at the next meeting of the association, methods applicable under American conditions to the official examination of basic slag phosphates.

Adopted.

(2) That the referee make a further study of methods for the preparation of neutral ammonium citrate.

Adopted.

(3) That the referee investigate the amount of wash water to be employed in the treatment of the residue from the ammonium citrate digestion.

This recommendation was amended to include a study of the manner of filtering and was so adopted.

#### INORGANIC PLANT CONSTITUENTS.

It is recommended—

(1) That the method for the separation of iron and aluminum offered as an official method be referred to the referee for 1909 for final recommendation. (See p. 93.)

Adopted.

(2) That further work be done on the sodium peroxid method for the determination of total sulphur in plants and plant products (Bulletin 107, p. 23).

Adopted.

#### SOILS.

It is recommended—

(1) That the modified J. L. Smith method for total potassium be adopted as a provisional method and be further studied. (Circular 32, p. 4.)

This recommendation was adopted in the modified form, as presented by the committee, the referee having recommended its adoption as an optional method.

(2) That the sodium peroxid fusion for total phosphorus be continued as a provisional method and be further tested. (Bulletin 105, p. 145.)

This recommendation also was adopted in the form presented by the committee, the referee having recommended the adoption of the method as official.

(3) That the magnesium nitrate method for total phosphorus be adopted as a provisional method and be further tested. (See p. 115.)

Adopted.

(4) That the Knorr method for the determination of carbonates in soils be further studied. (Wiley's Principles and Practice of Agricultural Analysis, vol. 1, ed. 1894, p. 338; ed. 1906, p. 380.)

Adopted.

#### CONVERSION TABLES.

Five conversion tables, submitted by W. J. Gascoyne, of Baltimore, Md., for the consideration of the association, were referred to Committee A, which recommended that the whole question of the adoption of such tables be referred to a special committee, and after some discussion the matter was referred to the standing committee on revision of methods.

## REPORT OF COMMITTEE ON FERTILIZER LEGISLATION.

The report of the committee of last year could not be sent out to interested parties until Saturday, November 7, 1908. This, of course, rendered it impossible to get any definite statements respecting the tentative definitions of fertilizers and of misbranding and adulteration. A number of replies have been received, however, a few of which, representing their general tenor, are submitted.

In the circumstances, therefore, the committee begs to report that it is desirable to postpone further action in regard to this important matter until the next meeting of the association. By that time the views of state officials, manufacturers, and farmers on the tentative definitions, etc., can be received and fully digested and placed in shape for consideration. The committee therefore recommends that it be continued with this report of progress for the purpose of a further study, in view of the criticisms which may be received on the definitions which have been submitted.

H. W. WILEY.

H. B. McDONNELL.

B. B. ROSS.

[The tentative definitions referred to in the report are as follows, together with extracts from the comments on the same received at the time of the meeting.]

### TENTATIVE DEFINITIONS OF FERTILIZERS AND OF MISBRANDING AND ADULTERATION.

(1) A fertilizer shall be defined as any simple, compound, or mixed material, prepared for the purpose of selling, or sold, or offered for sale, to be applied to the soil as nourishment for plants, or as a modifier of the soil in any respect in its relation to the growth of plants. The term "fertilizer material" (or ingredients) shall include every plant-food material which is utilized, or intended to be utilized, in the manufacture, preparation, or mixing of the fertilizers defined above.

(2) A fertilizer, or fertilizer material (or ingredient), shall be deemed to be adulterated—

(a) If the percentage of any of its ingredients fall materially below the professed standard under which it is sold, whether this standard appear as a label upon the package or as a guaranty in any other way by the vendor thereof.

(b) If any of the ingredients thereof have an origin other than that indicated upon the package, or guaranteed in any other way by the vendor thereof.

(c) If any of the ingredients of the fertilizer, or fertilizer material, be in a state of combination different from that indicated by the label or guaranteed by the vendor thereof.

(3) A fertilizer, or fertilizer material, shall be deemed as misbranded:

(a) If any false name or misleading statement or design or device be affixed to any package thereof or used in any way as a representation of the materials thereof by the vendor.

(b) If any false or misleading statement respecting the origin of the material be made upon the label, or any statement or guaranty of the vendor.

(c) If any false or misleading statement be made upon the label, or by the vendor, respecting the country or origin of the materials of which the fertilizer is composed.

(d) If any false or misleading statement be made on the label, or by the vendor, respecting the virtues or qualities of the fertilizer or the materials composing it.

(e) If sold under any false name or appellation, whether such name appear upon the package or label or be given to the article by the vendor thereof.

(f) If it be an imitation of or offered for sale under the name of another fertilizer or fertilizer material.

### COMMENTS.

(1) Definition (1) includes land plaster, ground limestone, etc. I would hardly favor the including of such materials, as any additional cost attached to such a substance as ground limestone would operate seriously against its use. In this State we do not consider ground phosphate rock (raw rock) among the commercial fertilizers, although its sale is in no way restricted.

(2) Would not the association do well to set a limit, or at least to suggest a limit, for each of the important plant-food elements, below which a guaranteed constituent would be considered as "materially" low?—C. A. MOOERS, *Tennessee Station*.

I have no suggestion or criticism to make with regard to the "tentative definitions of fertilizers and of misbranding and adulteration." They cover the ground fully to my mind. It is impossible to so frame definitions that there will not be a chance for difference of opinion as to what constitutes "misleading statement, or design, or device," and the best a law can do, it seems to me, is to lay down the general principles and leave it to the judgment of the individual officer in charge of the inspection work to decide whether the law in special cases has been violated as to misbranding. The recommendation of the committee as to work in the future would seem to be along the lines that give most promise of carrying the matter to a successful issue.—F. W. WOLL, *Wisconsin Station*.

Owing to the brief time in which to make a reply, I have only a few suggestions to make.

Definition 1 is more comprehensive than in most of the state fertilizer acts, and is evidently framed to include not only all materials sold as fertilizer, but, as well, all amendments. I confess I have considerable doubts about the wisdom of legislation so comprehensive at this time lest it too greatly encumber purely domestic exchanges. My present inclination is to prefer rather a law that takes into account only what are commonly recognized as commercial fertilizers.

I realize that there is considerable interstate traffic in certain lime products, as amendments, and that there is a possibility that abuse may spring up in this trade, but could it not be reached specifically rather than indirectly by including all amendments. The definition proposed is so broad that a carload of sand becomes a fertilizer if the sand is to be applied to affect the condition of the soil. The same is true of a carload of coal ashes applied for purely physical effects.

I do not believe it is a wise principle to enact police legislation far beyond present needs.

In making the above statements, I realize that the definitions for adulteration and misbranding which follow definition 1 are such that the objections I have offered to the definition for the word "fertilizer" may seem to be unnecessary, but I take it that if these definitions are adopted it will be for the purpose of advocating their incorporation in future state and national legislation, and that, in such setting, they will be accompanied by other clauses specifying the fertilizer materials that must be matters of guaranty. As soon as such clauses are introduced, the embarrassments I have in mind are likely to appear.

I desire to add that, in my judgment, the association, which is not specifically an organization charged with the execution of fertilizer acts, should not undertake the formulation and recommendation of a national fertilizer law, at least, until a full and formal conference shall have been had with the fertilizer control executive officials of the several States.—WM. FREAR, *Pennsylvania Station*.

\* \* \* I certainly think this law would be an advantage, especially to the manufacturers, as there would be uniformity in all of the States. As the case is now many of the fertilizer manufacturers are required to get out separate printed matter for many of the States. However, I do not believe the control of sale of fertilizers should go outside of the State in which sold, as I believe it can be looked after much better by men who are in close proximity to the places where fertilizers are handled. \* \* \*—T. L. CALVERT, *Ohio Department of Agriculture*.

On most of the recommendations I am heartily in accord with the views of the committee. In section 1, which defines a fertilizer and fertilizer material, it seems to me that some specific exemptions should be made. For instance, there are on sale in this State for the purpose of soil improvement prepared lime, limestone, land plaster, and marl, and hence our law expressly exempts barnyard manure, lime, wood ashes, and plaster when sold under their respective names. From the definition prepared by the committee there would no doubt arise the question as to whether such materials should not properly be included under a fertilizer law based on this definition. This point may not be well taken. I merely offer it for your consideration.

In a under 2 the question would naturally arise as to what is meant by "materially under the guaranty," and the interpretation of this term would be left solely to the judgment of the official in charge of fertilizer control. If it is possible to do so, I believe some definite statement as to what should be considered a material deficiency in any ingredient should be made, such a statement being based on the guaranteed content. That is, if the fertilizer was guaranteed to contain a certain percentage of ammonia, available phosphoric acid, and potash, a deficiency exceeding a certain per



cent of the guaranty would be considered as indicating intent to defraud.—W. J. JONES, Jr., *Indiana Station*.

\* \* \* My main criticism, other than those which are incorporated in the reprint as emanating from me, would be as to 2a. I believe that there should be added a proviso as follows:

“*Provided*, That if there should be a sufficient excess of other ingredients over the guaranty statement to make good the commercial equivalent of the promised plant food, the material may not be deemed adulterated.” You will notice that I have put the verb in the permissive rather than the mandatory form, so as to leave it in the discretion of the inspecting officer to say whether the proviso should or should not hold in a given case. I should strongly urge, however, before any goods are branded as adulterated under this act, that resampling and reanalyzing should be resorted to.—J. L. HILLS, *Vermont Station*.

We are heartily in favor of the enactment of a national fertilizer-control law, that would furnish a broad, scientific, and economical guide on this subject to state law-makers. The law should provide for actual experiments so that the relative value of plant food from all sources could be accurately shown without prejudice. The state system of fertilizer control is all wrong, for the reason that the men who frame the laws have not a sufficient knowledge of the subject. \* \* \* The great need of both the fertilizer industry and agriculture is positive knowledge without selfish influence or opinions not founded on facts. Terms should not be misleading, and the source from which the plant food is derived should be plainly stated. To leave these questions to the officials of the various States and the fertilizer manufacturers is a case of allowing the tail to wag the dog, and will prove a very unsatisfactory guide.—AMERICAN REDUCTION COMPANY.

#### REPORT OF COMMITTEE ON THE REVISION OF METHODS.

By J. K. HAYWOOD, *Chairman*.

The committee on the revision of methods presents as its report Bulletin 107, Revised, which was issued in July, 1908. The committee was empowered at the last meeting to make such changes in their first revision (Bul. 107) as were necessary to coordinate the methods and eliminate obsolete procedures. Such changes, together with the correction of typographical and other errors in Bulletin 107, were made in issuing the final revision. In submitting this report, I wish to thank the members of the committee and all those who have cooperated in the work, much patient and detailed labor having been put on it.

The report was accepted and a vote of thanks passed in recognition of the thorough manner in which the committee had discharged its office.

#### REPORT OF COMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By B. B. ROSS, *Chairman*.

##### MEDICINAL PLANTS AND DRUGS.

It is recommended—

(1) That the present provisional method for assaying opium be made official. (Bul. 107, Rev., p. 201.)

Adopted.

(2) That the methods included in the referee's report be made provisional.

Adopted. (These methods were made provisional in 1907, and are only slightly modified in this year's report. (See p. 129.)

(3) That the method outlined in this year's report for acetanilid mixtures be further tested, and that additional mixtures be tested by this and such other methods as may be found desirable. (See p. 100.)

Adopted.

(4) That macroscopical and microscopic methods for examining drug products be studied during the coming year.

Adopted.

(5) That microchemical methods for the identification of alkaloids in drug products be further studied.

Adopted.

(6) That other microchemical methods be tested to determine the possibility of thus identifying medicinal plant principles.

Adopted.

(7) That pharmacological methods for testing the quality of drug products be investigated.

Adopted.

(8) That two associate referees on medicinal plants and drugs be appointed for the ensuing year.

Adopted.

#### SUGAR.

It is recommended—

(1) That the question of the influence of precipitants upon the polarization of sugars be further investigated.

Adopted.

(2) That the question of methods of determining moisture or dry substance be further investigated, giving special attention to the method suggested by W. D. Horne and reported in the proceedings of 1907 (Bul. 116, pp. 22-23).

Adopted.

(3) That the method of determining dry substance by means of a refractometer and Geerligs' table be adopted provisionally.

Adopted.

(4) That under the method for the determination of copper contained in the precipitate of cuprous oxid, pp. 51-53, Bulletin 107, Revised, limit section (6), "Direct Weighing of Cuprous Oxid," page 53, by the following insertion: "This method should not be used in determining reducing sugars in commercial products, as other substances are precipitated along with the cuprous oxid. In these products the copper of the cuprous oxid should be determined direct by titration as in Low's method (Bul. 107, Rev., p. 241) or as cupric oxid."

Referred to the referee for 1908-9 for investigation, with the further recommendation that the term "commercial products" be more closely defined.

#### FOODS AND FEEDING STUFFS.

It is recommended—

That the referee for 1908-9 take up the question of acidity in cattle feeds and consider how the results obtained by current methods can be applied to agricultural problems.

Adopted.

#### DAIRY PRODUCTS.

It is recommended—

(1) That the following methods given in the referee's report (p. 153) for the analysis of condensed milk be adopted as official, namely: (1) Preparation of sample; (2) total solids; (3) ash; (4) protein; and (5) lactose.

These methods were referred to the referee for 1908-9 for final recommendation and action by the association as to their adoption as official.

(2) That the methods for the determination of sucrose in condensed milk by inversion with citric acid and by inversion with hydrochloric acid be investigated by the referee for the ensuing year.

Adopted.

(3) That the determination of fat in condensed milk be studied, special attention being given to solutions of less than 20 per cent concentration.

Adopted.

(4) That the New Babcock standard, proposed by E. B. Holland and referred to Committee B, be referred to the referee for 1908-9.

Adopted.

This contemplated standard<sup>a</sup> is as follows:

#### NEW BABCOCK STANDARD.

**SECTION 1.** The unit of graduation for all Babcock glassware shall be the true cubic centimeter (0.998877 gram of water at 4° C.).

(a) With bottles, the capacity of each per cent on the scale shall be two-tenths (0.20) cubic centimeter.

(b) With pipettes and acid measures, the delivery shall be the intent of the graduation and the graduation shall be read with the bottom of the meniscus in line with the mark.

**SEC. 2.** The official method for testing Babcock bottles shall be calibration with mercury (13.5471 grams of clean, dry mercury at 20° C., carefully weighed on analytical balances, to be equal to 5 per cent on the scale), the bottle being previously filled to zero with mercury.

**SEC. 3.** Optional methods: The mercury and cork, alcohol and burette, and alcohol and brass plunger methods may be employed for the rapid testing of Babcock bottles, but the accuracy of all questionable bottles shall be determined by the official method.

**SEC. 4.** The official method for testing pipettes and acid measures shall be calibration by measuring in a burette the quantity of water (at 20° C.) delivered.

**SEC. 5.** The limit of error: (a) For Babcock bottles, it shall be the smallest graduation on the scale, but in no case shall it exceed five-tenths (0.5) per cent, or for skim milk bottles one hundredth (0.01) per cent.

(b) For full quantity pipettes, it shall not exceed one-tenth (0.1) cubic centimeter, and for fractional pipettes five-hundredths (0.05) cubic centimeter.

(c) For acid measures, it shall not exceed two-tenths (0.2) cubic centimeter.

#### REPORT OF COMMITTEE ON RESOLUTIONS.

By L. L. VAN SLYKE, *Chairman.*

(1) *Resolved*, That we express to Professor Snyder our appreciation of the able and courteous manner in which he has presided over the deliberations of the convention.

(2) *Resolved*, That whereas a national bill to regulate the composition and sale of insecticides and fungicides has been recently drawn up by a committee composed of members of the Association of Economic Entomologists, manufacturers of insecticides, and agricultural chemists interested in insecticide and fungicide analysis, which bill will be presented to Congress for approval and passage; the Association of Official Agricultural Chemists does hereby express its approval of national legislation on this subject, which legislation it is believed will be of inestimable service in protecting the farming community as well as the legitimate manufacturer and in unifying state insecticide and fungicide laws.

The report of the committee was approved.

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<sup>a</sup> For further discussion of this standard, see Twentieth Annual Report of the Massachusetts Agricultural Experiment Station, January, 1908, p. 113.

APPOINTMENT OF COMMITTEE ON THE REVISION OF METHODS  
AND RECOMMENDATIONS OF REFEREES.

Mr. BIGELOW. It seems best in appointing this permanent committee to consider first the members of the editorial committee which had charge of the revision of the methods last year, and to appoint those who have so served for a short time and the newer members for a longer period. With this in view I will appoint—

To serve one year, J. K. Haywood, F. P. Veitch, and L. M. Tolman.

To serve two years, J. P. Street, F. W. Woll, and A. L. Winton.

To serve three years, B. B. Ross, E. M. Chace, and C. D. Howard.

Mr. Haywood will serve as chairman of the whole committee, which is to be subdivided as follows:

Committee A—Messrs. Haywood (chairman), Street, and Ross.

Committee B—Messrs. Woll (chairman), Veitch, and Chace.

Committee C—Messrs. Winton (chairman), Tolman, and Howard.

Mr. W. H. Bowker spoke at some length, urging the association to adopt some method for the estimation of available potash, as had been done in the case of available phosphoric acid, calling attention to the necessity for such action in conserving valuable by-products and furthering the interests of economic agriculture. The importance of this question had been already recognized by the association by creating a special refereeship for the consideration of the question of available potash, as recommended by the chairman of Committee A.

The place and time of meeting for the convention of 1909 was referred to the executive committee.

As it appeared from the special programme prepared for Monday that nearly all of the papers were on the subject of food adulteration, it was moved and carried that the association meet as a whole, not in sections, as originally contemplated.

The association adjourned to meet at 9 o'clock on Monday.

## FOURTH DAY.

### MONDAY—MORNING SESSION.

The association convened at 9 o'clock for the reading of special papers in accordance with the resolution adopted in 1907. Mr. Tolman, as chairman of Section C, presided.

#### METHODS RELATING TO THE RATE OF DECOMPOSITION OF ORGANIC MATTER IN THE SOIL.

By JACOB G. LIPMAN.

Chemically considered, humus is a comparatively inert substance; biologically considered, it is readily susceptible to a wide range of modification. The host of bacteria, fungi, and yeasts that inhabit it find no difficulty in inducing its transformation, which, in turn, reacts on the growth of crops. In so far as the bacteria and other microorganisms of the soil find suitable conditions of moisture, temperature, aeration, and chemical constitution, the humus will decay rapidly. In so far as these conditions are unsuitable, the decomposition will be slow; and the supply of available nitrogen, and probably of phosphorus and potassium also, will be but meager. This fact was well appreciated even before the function of bacteria in the soil was recognized, as may be seen, for instance, from some experiments by Boussingault and Loewy <sup>a</sup> published in 1853.

The recognition of humus as an important factor in crop production <sup>b</sup> has led logically to the analytical study of its decomposition products and their quantitative determination. Carbon dioxid, ammonia, and nitrates seemed important among these decomposition products not merely because of their indirect or direct action as sources of plant food, but because of their value as indicators of quantitative reactions. Students of soils tried to establish a possible relation between the productive power of soils and their content of carbon dioxid, ammonia, or nitrates. We need only mention here in passing the investigations of Boussingault and Loewy, <sup>c</sup> and of von Fodor, <sup>d</sup> as bearing on the presence of carbon dioxid in soil air; the rather careful work of Baumann <sup>e</sup> on the determination and presence of ammonia in soils; and the examination by Boussingault <sup>f</sup> of various soils for their content of nitrates.

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<sup>a</sup> Mémoire sur la Composition de l'Air Confiné dans la Terre Végétale, Ann. chimie physique, 1853 (3), 30:3.

<sup>b</sup> Liebig, Die Chemie in ihrer Anwendung auf Agricultur und Physiologie, 9th ed., Braunschweig, 1876, p. 26.

<sup>c</sup> Loc. cit.

<sup>d</sup> Deutsche Vierteljahrsschrift öffentl. Gesundheitspflege, 1875, 7:205-237.

<sup>e</sup> Ueber die Bestimmung des im Boden enthaltenen Ammoniak-Stickstoffes und über die Menge des assimilierbaren Stickstoffes im unbearbeiteten Boden, Habilitationsschrift, Berlin, 1886.

<sup>f</sup> Agronomie, chimie agricole et physiologie, 3d ed., 2:40.

The evidence gathered by these earlier investigators shows a distinct, though not always uniform, relation between the productive power of soils and their content of carbon dioxid, of ammonia, or of nitrates. We note that the air of fertile soil usually contains more carbon dioxid than the air of unproductive soil. Similarly, the fertile soils contain, as a rule, more ammonia and more nitrate than unproductive soils. But even admitting this, it seems hardly practicable to draw definite conclusions as to the future behavior of a soil from its content of the substances in question. The amount of carbon dioxid in the soil air is an indication of oxidation changes already accomplished, but not necessarily a guide to future oxidation intensity. The organic constituents of the humus, as well as the character of the microorganisms, may have been modified to preclude rapid oxidation. In the same way the quantity of ammonia in the soil is only a measure of past performance, and a very inadequate measure at that. As a transition product ammonia may be speedily oxidized to nitrates, or it may be transformed into protein substances by plants or fungi. Hence the quantity of ammonia present at any time in cultivated soil can not even serve to indicate past intensity of ammonia formation. As to nitrates, they, too, are not stable in the soil. Like ammonia, they may be utilized by higher plants, or by bacteria, yeasts, and molds for the production of new protein compounds. They may likewise be destroyed by denitrifying bacteria, or they may be leached out of the soil by excessive rainfall. In a word then, the amounts of carbon dioxid, ammonia, and nitrates in field soil are but an incomplete measure of past performance and a very inadequate guide as to future efficiency.

The better understanding of the functions of humus, which has gradually come in the wake of bacteriological investigations, has suggested new methods for the study of organic matter and its transformation in the soil. In experiments like those of Wollny,<sup>a</sup> or in the more recent experiments of Stoklasa and Ernest,<sup>b</sup> the evolution of carbon dioxid from soils kept under definite experimental conditions has been employed as a measure of the activities of the soil bacteria and of the susceptibility of the humus to decay. The same purpose has been accomplished in the experiments of Russell,<sup>c</sup> and of Darbishire and Russell,<sup>d</sup> by measuring the absorption of oxygen instead of the evolution of carbon dioxid. These methods enable us, therefore, to study the possible future behavior of the humus compounds under given conditions. In other words, we are enabled to secure some information concerning the relative availability of the constituents in the soil humus. For instance, it was found by Stoklasa and Ernest in a comparison of several soils that the average daily production of carbon dioxid in 1,000 grams of soil ranged from 17.5 to nearly 60 milligrams. More carbon dioxid was produced by the soil than by the subsoil, the aerobic activities being more prominent in the former, the anaerobic activities in the latter. Similarly, at 35° C. about twice as much carbon dioxid was produced as at 20° C. Darbishire and Russell found that in a number of untreated soils examined the absorption of oxygen in nine days ranged from 6 to 27 millimeters.

Analogous attempts at measuring the rate of decay of soil humus and of other organic materials have been made, not by determining the oxidation products of the carbon but of the nitrogen in the soil humus. It was well known that ammonia almost invariably appears as one of the products in the oxidation of nitrogenous materials of organic origin. It was likewise recognized after the convincing experiments of Müntz and Coudon<sup>e</sup> that ammonia formation in the soil is a biological

<sup>a</sup> J. Landwirtsch., 1886, 34 : 222.

<sup>b</sup> Centrbl. Bakt. Para., 1905, pt. II, 14 : 723; also Zts. Zuckerind., Böhmen, 1907, 31 : 291.

<sup>c</sup> J. Agr. Sci., 1905, 1 : 260.

<sup>d</sup> Ibid., 1907, 2 : 305.

<sup>e</sup> Compt. rend. acad. sci. Paris, 116 : 395.

process and distinct from nitrification proper. Further information was supplied by Marchal <sup>a</sup> in his demonstration of the intense oxidizing activities of *B. mycoides* involving the formation of carbon dioxide and of ammonia. It was perceived at the same time that the quantitative estimation of ammonia in the soil could lead to no definite conclusion because of the further changes which ammonia undergoes in the soil. The same may be said also of the quantitative estimation of nitrites.

On the other hand, the determination of nitrates in soils kept under definite conditions promised to give valuable information not only as regards the rate of decomposition of the soil humus, but also as regards the availability of various nitrogenous fertilizers. It is not surprising, therefore, to find in agricultural literature a vast amount of data bearing on the formation and accumulation of nitrates in the soil.<sup>b</sup> We owe to these investigations a broader point of view and a deeper insight into conditions of soil, climate, and cropping in so far as they affect the oxidation of organic matter in the soil.

The many interesting facts brought to light by various nitrification experiments served to emphasize, among other things, the necessity of distinguishing the individual factors more or less prominent in the formation of nitrates. It seemed evident that, apart from conditions of moisture and temperature, the process of nitrification is directly affected by at least three important factors, viz, the physical and chemical composition of the inorganic constituents of the soil; the physical and chemical composition of the organic constituents of the soil; the character of the nitrifying, and perhaps of other bacteria present in the soil. Without going far afield, we may note in this connection the interesting experiments of Withers and Fraps.<sup>c</sup>

We find, in the first place, decided differences in the rate of oxidation of substances like dried blood, cotton-seed meal, dried fish, tankage, bat guano, bone, and ammonium sulphate. Not only were these differences maintained, but they were in more or less close agreement with the corresponding differences brought out by digestion tests and vegetation experiments. We find also that the same nitrogenous substances were nitrified to a very unequal extent in different soils.<sup>d</sup> For instance, in five different soils the proportions of nitrate nitrogen formed from cotton-seed meal under the conditions of the experiment were 4.4, 17.6, 22.9, 41.2, and 54.8 per cent, respectively. Evidently there were wide divergences in the physical, chemical, and bacteriological make-up of these soils.

But, interesting as are the facts just noted, we encounter in the work of Withers and Fraps <sup>e</sup> a fact which is even more significant in its bearing on the physiology of nitrification, namely, that in different soils ammonium sulphate and cotton-seed meal are not nitrified in the same order. The authors are therefore led to conclude that there may exist in the soil an organism or organisms capable of oxidizing organic matter (they should have said ammonia) directly to nitrites or nitrates. This assumption has been strengthened since by the investigations of Kaserer,<sup>f</sup> who believes he has found an organism capable of changing ammonia directly into nitrate.

It would hardly be safe to theorize too much with these meager facts as a basis, but for our purpose we may accept them at their face value in so far as they tend to show the need of differentiation in the study of decay processes. The nitrates present in the soil at any time may be but a small fraction of the total amount ac-

<sup>a</sup> Bul. soc. belge microsc., 1893, p. 83.

<sup>b</sup> U. S. Dept. Agr., Office of Experiment Stations, Bul. 194, p. 57.

<sup>c</sup> North Carolina Agr. Exp. Sta., Bul. 176, p. 19.

<sup>d</sup> North Carolina Agr. Exp. Sta., Report of the Chemist, 1902-3, p. 6.

<sup>e</sup> North Carolina Agr. Exp. Sta., Annual Report, 1901-2, p. 37.

<sup>f</sup> Centrbl. Bakt. Para., 1906, 16 [2] : 681.

tually produced. It is well known that processes are constantly at work in the soil unfavorable to the accumulation of nitrates. Entirely apart from possible losses by leaching, there is the more or less remote but still real danger of denitrification. In addition to this there is the constant draft on the store of soil nitrates by bacteria, molds, yeasts, and algae, not to mention higher plants when these are included in the experiment.

In view of these facts the more recent investigations on the decay of organic matter in the soil frequently attempt at least a partial differentiation of the single stages of the process. I am not aware of systematic attempts to determine albumoses and peptones among the fragments of protein decomposition in the soil. There are, however, systematic studies of ammonia formation as something independent of nitrite or nitrate formation. Indeed, we have come to accept the term ammonification (or ammonization) as expressing a definite change or series of changes. However, before taking up the discussion of methods relating to the study of ammonification, nitrification, and denitrification in the soil itself, it would be proper to consider here certain methods <sup>a</sup> which deal with the same reactions from a somewhat different standpoint.

The methods in question are based on the changes which occur in solutions of known composition when inoculated with a given weight of soil. For instance, a sterile solution of peptone or of gelatin, when inoculated with soil, will undergo decay, and a portion of the organic nitrogen will be split off as ammonia, which can be readily distilled off and estimated. Now, it happens that in equal quantities of the same solution inoculated with equal weights of different soils the amounts of ammonia produced may differ widely. Otherwise stated, soils vary in their ammonifying power. But since ammonification is a biological process, we are forced to the conclusion that the differences noted are due either to the unequal numbers of bacteria introduced into the sterile solutions by the different soils, or to differences in the species or vigor of the organisms, or perhaps to both. Be it as it may, the ammonification coefficients show fairly constant characteristics bearing a more or less definite relation to the productive capacity of the corresponding soils. Similarly, solutions have been prepared to favor the growth of nitrifying, denitrifying, or nitrogen-fixing bacteria looking toward the determination of the nitrifying, denitrifying, or nitrogen-fixing coefficients of soils. The latter methods have not, on the whole, proved as consistent in their results as have the ammonification methods. However, it would be out of place here to discuss them in detail, particularly since they have been considered elsewhere.<sup>b</sup>

On the other hand, it would be well worth while to consider here the fundamental differences between the methods just outlined and those based on the study of bacteriological processes in the soil itself. We can well appreciate, of course, how 10 grams of one soil might cause the production of more ammonia in peptone solutions than 10 grams of another soil under identical experimental conditions. One soil might have two or three times as many ammonifying bacteria as another; or it might have not only larger numbers, but also species and individuals with a particularly well-developed power of ammonia formation. Moreover, it appears quite logical to assume that large numbers and vigorous species may produce large quantities of ammonia in the soil itself as well as in the culture solutions. Hence the analogy between the changes in suitable culture solutions and the returns from pot or field experiments.

Theoretically, however, this analogy could not always be expected to exist. It must be remembered that we are dealing here with micro-organisms entirely detached

<sup>a</sup> U. S. Dept. Agr., Office of Experiment Stations, Bul. 194, p. 10.

<sup>b</sup> New Jersey Agr. Exp. Sta., Bul. 210; Annual Report, 1905, p. 225; 1906, p. 119; 1907, p. 186.



from their normal medium, the soil. By placing a portion of the latter in an artificial culture solution we create entirely new conditions for the growth of the bacteria. Free play is given thereby to the establishment of new group relationships, and species obscure in the soil itself may come to the front. Hence it may often happen, as it often *does* happen, that the ammonification coefficients of two soils, as indicated by experiments in solution, "umsetzungsversuche," as the Germans would call them, do not at all correspond to actual conditions. We must therefore distinguish here between ammonifying *power* as referring to the numbers and species of the bacteria themselves, and what Fraps<sup>a</sup> has recently designated as ammonifying *capacity*, as referring to the physical and chemical constitution of the soil as well as to the number and species of its bacteria.

The differentiation of the various bacteriological changes in the soil itself is not a simple matter. As already noted, the activities of the decay bacteria in the soil can not be measured by estimating the quantity of ammonia formed under normal conditions. The ammonia nitrogen does not accumulate, for reasons already noted. We can, however, create conditions in the soil precluding the further oxidation of ammonia. This may be accomplished by the addition to the soil of a sufficient quantity of dextrose or of other soluble carbohydrates, or salts of certain organic acids. The same purpose may be achieved, perhaps, by skillfully adjusting the reaction of the soil. By these means the ammonifying bacteria are permitted to grow while the nitrifying bacteria are suppressed. The ammonia accumulates in the soil and may be readily estimated.

The defect of this method lies in the fact that the formation of ammonia from the soil humus is, analytically, a comparatively slow process. A further defect is due to a probable rearrangement in the numbers and kinds of the decay bacteria, due to the materials added or the artificial conditions created. As will be seen presently, the first of these defects may be remedied without difficulty. The second can not be eliminated so easily, yet is not necessarily fatal to the successful application of the method.

Ammonia formation in the soil may be greatly intensified and the simultaneous suppression of nitrification effected in still another way. By mixing with the soil certain quantities of peptone, of urea, or of other nitrogenous organic substances we supply to the bacteria something from which ammonia may be produced readily and in comparatively large quantities. At the same time, the presence of these substances stops the growth of the nitrifying bacteria. In the practical application of this method in our laboratory we thoroughly mix 0.5 gram of peptone or 0.25 gram of urea with 100 grams of fresh soil, transfer the mixture into a beaker, adjust the moisture content by the addition of sterile water, cover the beaker with a glass dish, and place it in the incubator or closet. We usually sterilize the beaker, peptone, and urea before they are brought in contact with the soil. The latter is drawn with the customary precautions against gross contamination. At the end of three or four days the contents of the beaker are transferred to a 2-liter copper flask, about 150 cc of water added, and a sufficient quantity of magnesium oxid. The distillation and titration of the ammonia are performed in the customary manner.

Similarly, we may study nitrate formation in the soil itself by placing weighed quantities of the latter in beakers and maintaining suitable moisture and temperature conditions. At the end of four weeks (or of longer intervals, if desired) the soil is leached and the nitrites and nitrates determined in the leachings. In order to intensify the nitrification processes we may add to the soil weighed quantities of ammonium salts or of organic nitrogenous substances. The quantities of nitrate, which is the end product of various bacteriological activities, may serve to gauge the comparative rate of oxidation of the organic matter. This method may be employed—has, indeed,

<sup>a</sup>Texas Agr. Exp. Sta., Bul. 106.

been repeatedly employed—for the study of the comparative availability of different nitrogenous substances as a source of nitrogen to plants.

Comparative studies of denitrification and nitrogen-fixation may be made by the same method. It is merely necessary to modify the cultural conditions by the addition of certain substances. In the case of denitrification, for instance, we add a known amount of potassium or sodium nitrate, leach the soil at the end of ten days, and determine the ammonia, nitrite, and nitrate nitrogen in the leachings and the total nitrogen in the residue. The initial nitrogen content of the soil being known, we have the complete data required.

The methods just outlined may be still further differentiated. We may find means to distinguish the single phases of ammonification as due to urea bacteria, spore or nonspore-forming aerobes, spore or nonspore-forming anaerobes. In the case of nitrification, we may attempt to distinguish the single phases of oxidation; in the case of denitrification the single phases of reduction; in the case of nitrogen-fixation the aerobic and anaerobic phases of the process. The applications suggested may enable us to gain an insight into the decay processes in the soil, which are imperfectly understood at present. Moreover, we shall not only gain in our ability to interpret past reactions as revealed by analysis, but also be enabled to forecast future reactions and quantitative changes of importance to plant food production and its assimilation by the growing crop.

An interesting paper on the determination of sulphurous acid and sulphites or sulphur dioxid in food products was submitted by Mr. Edward Gudeman. The paper comprised a comparison of the method adopted by the association and a modified method suggested by the author, the modification consisting in driving over the volatile products with low-pressure steam rather than by direct distillation, as in the association method. The steam is generated from distilled water and passed directly into the mass through a glass U-tube. The details of the paper are to be found in the *Journal of Industrial and Engineering Chemistry* for February, 1909.

#### THE POSSIBILITIES OF MUSCOVADO SUGAR AS AN ADULTERANT FOR MAPLE PRODUCTS.

By R. E. DOOLITTLE and A. F. SEEKER.

Occasionally there have been presented for entry at the port of New York shipments of a brown-colored sugar from Venezuela designated as "Melada" or "Melado." The product is generally in the form of rectangular cakes about 1 inch thick by 5 inches long by 4 inches wide. The cakes vary somewhat in color, but in general closely resemble maple sugar in appearance. Their use as an adulterant or substitute for the maple product seemed quite probable, and the finding of a large quantity of this grade of sugar in the factory of a dealer in maple products by one of our inspectors showed the necessity of making a careful examination of the product. We were surprised to find on employing the usual methods for determining the purity of maple sugar that the brown sugar gave practically the same results as does pure maple sugar. These figures, together with those of a pure maple sugar run at the same time, are given in the table:

## Composition of muscovado and maple sugars.

Determination.	N. Y. 10676, light muscovado sugar.	N. Y. 10677, dark muscovado sugar.	I. S. 758-a, Vermont maple sugar.
Moisture (per cent).....	7.35	7.50	2.80
Ash (per cent).....	1.33	1.30	1.10
Polarization, direct, at room temperature (° V.).....	+80.0	+82.4	+81.0
Polarization, invert, at room temperature (° V.).....	-27.0	-26.8	-29.6
Polarization, at 86° (° V.).....	± 0.0	± 0.0	± 0.0
Sucrose (Clerget) (per cent).....	81.4	83.1	85.6
Winton lead number.....	2.08	2.12	2.26
Malic acid value.....	1.19	1.24	.....

An analysis of the ash, however, showed a distinct difference, as is shown by the following data:

## Analysis of the ash of muscovado and maple sugars.

Determination.	N. Y. 10676, light musco- vado sugar.	N. Y. 10677, dark musco- vado sugar.	I. S. 758-a, Vermont maple sugar.	Average of four samples of maple sugar. <sup>a</sup>
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Insoluble in boiling nitric acid (1:3).....	2.55	3.41	8.9	.....
Potassium oxid.....	50.08	49.89	23.6	26.49
Sodium oxid.....	4.85	2.32	1.6	.....
Calcium oxid.....	5.77	5.66	35.9	24.98
Magnesium oxid.....	2.20	2.63	3.0	.....
Ferric oxid.....	.29	.26	Slight trace.	.....
Chlorin.....	1.96	1.34	Trace.	.....
Sulphur trioxid.....	22.16	23.21	None.	1.82
Phosphoric acid.....	4.02	3.68	.45	.....
Undetermined.....	6.12	7.60	26.55	.....
Ratio $\frac{K_2O}{CaO} \times 100$ .....	868	881	66	106
Ratio $\frac{SO_3}{CaO} \times 100$ .....	384	410	.....	7
Ratio $\frac{SO_3}{K_2O} \times 100$ .....	44	47	.....	7
Ratio $\frac{P_2O_5}{CaO} \times 100$ .....	70	65	1	.....

<sup>a</sup> Jones, Eighteenth Annual Report, Vermont Agr. Exp. Sta., 1905, p. 331.

The ash of the brown sugar consists mostly of potassium sulphate, while over 80 per cent of that of maple sugar is composed of carbonate of potassium and calcium, these two bases existing in approximately equal parts. From these facts one is led to believe that a determination of water-soluble and water-insoluble ash, their ratio, and their alkalinity would furnish the necessary evidence as to whether the product under examination was composed of maple sugar or muscovado sugar. As a matter of fact, these determinations when carried out on one of the samples gave the results shown in the following table:

*Ash determinations and ratios of muscovado and maple sugars indicative of adulteration.*

[All results reduced to a moisture-free basis.]

Determination.	N. Y. 10677, muscovado sugar.	I. S. 758-a, maple sugar.	Maple sugars. <sup>a</sup>
Water-soluble ash (per cent).....	1.23	0.50	0.53
Water-insoluble ash (per cent).....	.17	.64	.48
Ratio water-soluble ash water-insoluble ash.....	7.7	.8	1.1
Alkalinity of water-soluble ash (cc tenth-normal acid).....	.11	.49	.68
Alkalinity of water-insoluble ash (cc tenth-normal acid).....	.03	1.47	1.01

<sup>a</sup> Average of a number of analyses made by Jones, Vermont Agr. Exp. Sta. Report, 1905.

Unfortunately a sirup prepared from the muscovado sugar fermented before time could be found to make the usual determinations, and no more of the sample remained for further work. However, it seems reasonable to assume that the sirup as well as the sugar could be detected by the high ratio of insoluble ash to soluble ash, and the low alkalinity of both. As confirmatory evidence an ash analysis should be made wherein a high percentage of potassium oxid and sulphur trioxid, together with a small amount of calcium oxid, would indicate the adulteration. It would appear also as if the phosphoric-acid content gave useful information, both of the muscovado sugars possessing notable amounts and the maple sugar little. These data are also given in the table in the form of ratios, which serve better to emphasize the contrast. As there is good reason to suspect that the brown sugar under discussion is being used by manufacturers of maple products, it seems highly important that an examination of the ash should be included in all routine analyses of these goods.

Acknowledgments are due to Mr. W. A. Bender for the ash analyses here given and to Mr. A. E. Taylor for many of the other determinations.

#### NOTES ON THE WINTON LEAD NUMBER OF MIXTURES OF CANE AND MAPLE SIRUP.

By R. E. DOOLITTLE and A. F. SEEKER.

Among a number of samples of cane and maple sirup mixtures examined during the past year were a few which had been mixed in the presence of one of the officials of the laboratory and were known to contain 10 per cent of maple sugar. Upon analysis it was found that these sirups gave no precipitate whatever with basic lead acetate when making the lead number determinations according to Winton's method.

A sample of the same maple sugar from which the sirups had been prepared was examined at the same time and gave a lead number of 2.31, besides other results which indicated the purity of the product, and therefore the negative results obtained with the 10 per cent mixtures caused some surprise.

Upon carefully repeating the determination it was observed that a precipitate was formed when the lead subacetate solution first came in contact with the sirup, but this was later redissolved when the whole of the reagent had been added. It was judged, therefore, that so great an excess of basic acetate prevented the usual precipitation with mixtures of this strength.

A number of portions of 5 grams each were accordingly taken, diluted to 15 cc with water, and placed in test tubes. To each of these were added different amounts of the standard basic acetate solution, varying from 0.1 to 5 cc, and after thorough shaking the turbidity noted. The tubes to which the smaller amounts of reagent had been added were perfectly clear, but a turbidity appeared as the quantity approached 0.5 cc, then came a slight precipitate, which reached its maximum at 1 cc and gradually decreased again to only a slight opalescence with 5 cc. Winton's method calls for 25 cc

of reagent for 25 grams of sugar or sirup, a proportion of 1 cc per gram of substance. The maximum precipitate was in this case produced by 1 cc to 5 grams of substance. A lead number determination was accordingly made on the 10 per cent maple sirup in question with a lead subacetate solution five times weaker than that prescribed by Winton, and the figure 0.137 obtained. This solution is much too weak to be used with pure maple sirups, as it contains only about 0.8 gram of lead (figured as metal) per 100 cc, whereas 100 grams of an average maple sugar will precipitate in Winton's method over 2 grams of lead. To show that a large excess of lead reagent is necessary to produce a normal precipitate, a sirup containing 30 per cent of cane sugar and 30 per cent of maple sugar was prepared and the lead number determined, using both the Winton solution and the one diluted five times. With the weak subacetate a lead number of 0.29 was obtained, with the strong, 0.72. In the former case there was just enough basic subacetate in the 25 cc of solution added to have precipitated all of the lead if a normal precipitation had occurred, and no lead would have appeared in the filtrate. Actually the amount of lead was insufficient for maximum precipitation and the lead number was accordingly too low. On comparing the amount of lead remaining in solution with that added it was seen that the former was 62 per cent of the latter. By the regular method the excess of lead producing a normal lead number was found to be 81.7 per cent. In the case of the 10 per cent maple sirup in which no precipitation was produced by the regular solution and in which a lead number of 0.137 was obtained with the 1 in 5 dilution, it was found that the excess of lead was 82.1 per cent. As a conclusion it would appear that at least 62 per cent excess of lead is necessary for a complete precipitation, an excess of lead much greater than 80 per cent tends to prevent precipitation, and that a zero lead number obtained by the regular method does not indicate that so-called cane and maple sirups contain no maple sugar.

It has been suggested that the lead number of the mixture containing 10 per cent of maple sugar might give normal results if the solution after addition of the lead reagent were allowed to stand longer than two hours, as was done in the previous determinations. On standing for twenty-four hours the opalescence which formed on adding the lead subacetate had collected into a very slight precipitate, which was matched in the blank by one of similar proportions, though boiled distilled water had been used in all cases. After filtering in the usual way and determining the lead number zero values were obtained as before.

### THE DETERMINATION OF FUSEL OIL BY ALKALINE PERMANGANATE.

By A. S. MITCHELL and C. R. SMITH.

Fusel oil consists chiefly of a mixture of normal and isopropyl, normal and isobutyl, active amyl and isoamyl, and hexyl alcohols. The Allen-Marquardt method is in reality an estimation in terms of amyl alcohol of the higher alcohols which are dissolved and retained by carbon tetrachlorid, under fixed conditions, and converted into volatile acids by oxidation with a chromic acid mixture.

This paper is the result of an effort to learn the conditions necessary to produce definite oxidation of the various alcohols by alkaline potassium permanganate solution. It was hoped to avoid prolonged digestion with the oxidizing agent and, later, the subsequent distillation with the attendant concentration of the oxidizing mixture. Ordinary amyl alcohol, which consists of iso and active amyl alcohols, was first experimented upon. In the first effort the manganese dioxid and unreduced permanganate was not removed after acidifying for the distillation of the free acids. The mixture bumped so badly that the distillation could not be completed. It became necessary to remove the manganese dioxid and permanganic acid. Oxalic acid was tried and rejected. Hydrogen peroxid was finally selected for this purpose.

In the experiments recorded in the following table the amyl alcohol used had been dried and fractionated at boiling points between 128° and 132° C. After the oxidation 50 cc of sulphuric acid (1:4) was added and then an excess of hydrogen peroxid, and the mixture was boiled under a reflux condenser for fifteen minutes to remove any carbon dioxide. The mixture was then distilled until bumping occurred due to the separation of salts from the solution; 80 cc of water were added and distillation repeated to the same point. The valeric acids were then titrated with tenth-normal sodium hydroxid with the following results:

*Amyl alcohol estimated under varying conditions, using alkaline potassium permanganate.*

Time of oxidation.	Total dilution during oxidation.	Temperature of oxidation.	Potassium hydroxid.	Potassium permanganate.	Amount of amyl alcohol.		
					Quantity used.	Quantity found.	Per cent found.
<i>Minutes.</i>	<i>cc.</i>		<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>	
30	60	Room.....	5	3	0.1170	0.1047	89.5
10	60	Boiling-water bath.....	5	3	.1347	.0959	71.2
10	60	do.....	5	3	.1190	.0833	70.0
20	60	Boiled, free flame.....	5	3	.1245	.0471	37.8
20	160	Room.....	5	2.5	.1080	.0960	88.9
20	160	Boiling-water bath.....	5	2.5	.1181	.0915	77.5
<i>a</i> 20	260	Room.....	5	2.5	.1259	.1093	86.8
10	160	do.....	5	2.5	.1161	.1040	89.6
10	160	do.....	5	2.5	.1090	.0903	82.9
30	160	0°.....	5	2.5	.1099	.1091	99.2
30	160	0°.....	5	2.5	.1156	.1126	97.4
30	160	0°.....	5	2.5	.1545	.1522	98.5
30	160	0°.....	5	2.5	.1631	.1566	95.4
30	160	0°.....	5	2.5	.1986	.1909	96.1

*a* Hours.

At a temperature of 0° C. the oxidation of amyl alcohol to valeric acid appears to be quantitative and at higher temperatures the yield of valeric acid is decreased. Variations in time within the above limits have little influence upon the reaction. When the oxidation is too vigorous destruction occurs and the results are low. If the reaction is controlled by decreasing the temperature the oxidation is arrested at the production of the corresponding acids. The acids when produced are not easily altered and may be allowed to stand with the oxidizing agent at room temperature without appreciable change.

#### THE OXIDATION OF NORMAL PROPYL AND ISOBUTYL ALCOHOLS.

Similar experiments were made upon normal propyl and isobutyl alcohols, following the same procedure as in the case of amyl alcohol, and with the following results.

*Estimation of propyl and isobutyl alcohols, using alkaline potassium permanganate.*

Alcohol.	Amount used.	Amount recovered.	
		Quantity found.	Percentage found.
	<i>Grams.</i>	<i>Grams.</i>	
Propyl.....	0.1151	0.1156	100.4
Do.....	.1151	.1190	103.4
Isobutyl.....	.1005	.0981	97.6
Do.....	.1005	.1039	103.4

**THE EXTRACTION OF PROPYL, ISOBUTYL, AND AMYL ALCOHOLS FROM ETHYL ALCOHOL SOLUTIONS.**

A few trials were made upon the extraction of these alcohols by carbon tetrachlorid. In each case 100 cc of 43 per cent by volume alcohol, containing known amounts of one of the alcohols, was diluted to 115 cc, salted, and saturated sodium chlorid solution added to bring the specific gravity to 1.10, extracted with four successive portions of 40, 30, 20, and 10 cc, respectively. The carbon tetrachlorid was washed with three portions of saturated salt solution 50 cc each, and with one portion of 50 cc of saturated sodium sulphate solution. The alkali was added to the carbon tetrachlorid solution contained in a separatory funnel and cooled in an ice bath to 0° C. The permanganate, previously cooled, was added and allowed to remain in the bath thirty minutes, with repeated shaking. The distillation was conducted as previously described.

*Extraction of alcohols, using carbon tetrachlorid.*

Alcohol.	Quantity used.	Quantity found.	Percentage found.
	<i>Grams.</i>	<i>Grams.</i>	
Propyl.....	0.1292	0.0225	17.5
Do.....	.1292	.0237	18.3
Isobutyl.....	.1620	.0920	56.7
Do.....	.1620	.0953	58.8
Amyl.....	.2025	.1747	86.2

Attempts to extract amyl alcohol from ethyl alcohol solutions, using petroleum ether as a solvent, failed.

**DIRECT VOLUMETRIC METHOD FOR THE DETERMINATION OF PROPYL, ISOBUTYL, AND AMYL ALCOHOLS.**

Repeated attempts have been made during the course of this work to estimate propyl, isobutyl, and amyl alcohols, depending on the amount of permanganate reduced, but without success until satisfactory conditions had been determined for the oxidation. The permanganate consumed was greatly in excess of the theoretical amount. The unused permanganate was determined by adding a known amount of oxalic acid in excess and titrating back with permanganate.

The alcohols were contained (or dissolved) in 50 cc of water, to which 10 cc of potassium hydroxid were added and the whole cooled in an ice bath. One hundred cc of an aqueous solution containing 2 grams of potassium permanganate and previously cooled in the ice bath were then added and the mixture was allowed to stand for thirty minutes. It was then removed from the bath, acidified with 50 cc of 24 per cent sulphuric acid, a known excess of oxalic acid was added; the mixture was then warmed and the excess of oxalic acid titrated back with standard permanganate. The following factors were used as calculated from the theoretical oxidation of the alcohols to their acids by potassium permanganate: One gram of permanganate equals 0.475 gram of propyl alcohol, or 0.585 gram of isobutyl alcohol, or 0.696 gram of amyl alcohol. The results follow:

*Preliminary trials for the volumetric determination of alcohols.*

Alcohol.	Amount used.	Amount found.	Per cent found.
	<i>Gram.</i>	<i>Gram.</i>	
Propyl.....	0.1150	0.1245	108
Do.....	.1150	.1246	108
Isobutyl.....	.1005	.1094	109
Amyl.....	.1482	.1910	130
Do.....	.1074	.1400	130

These figures seemed fair in the cases of propyl and isobutyl alcohols, but poor for amyl alcohol. They apparently indicate the complete destruction of a portion of the latter at some stage of the process. Varying the amount of alkali made no improvement.

That the results were slightly high, as in the case of the propyl and isobutyl alcohols, might be expected, for permanganic acid and manganese dioxid are produced on acidifying and are present together for several minutes during reduction by oxalic acid. The excessive destruction of permanganate with amyl alcohol points to other causes.

As oxalic acid acts very slowly in reducing the manganese dioxid, it was decided to substitute hydrogen peroxid. Sulphuric acid was added to hydrogen peroxid and the mixture resulting from the oxidization was poured slowly into it. This gives the permanganate little opportunity to react with free organic acids, as it is reduced by the hydrogen peroxid as it is acidified.

*Determination of amyl alcohol, substituting hydrogen peroxid for oxalic acid.*

Amyl alcohol used.	Perman-ganate consumed.	Amyl alcohol found.	Per cent found.
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	
0.1276	0.1850	0.1287	100.9
.1276	.1830	.1274	99.8
.1276	.1827	.1272	99.7
.1536	.2173	.1512	97.1
.2042	.2971	.2067	101.2

A series was run in which isobutyl and propyl alcohols were used instead of amyl alcohol.

*Determination of isobutyl and propyl alcohols, substituting hydrogen peroxid for oxalic acid.*

Alcohol.	Amount used.	Perman-ganate consumed.	Amount found.	Per cent found.
	<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	
Isobutyl.....	0.1005	0.1640	0.0959	95.0
Do.....	.1005	.1645	.0960	95.0
Do.....	.1407	.2240	.1300	92.4
Do.....	.1608	.2525	.1477	91.8
Propyl.....	.1150	.1625	.0770	67.0

The results indicate incompleteness of the oxidation at 0°, so the procedure was adopted of starting the oxidation at 0° and allowing the mixture to warm slowly to room temperature. The mixture was allowed to remain ten minutes in the ice bath and taken out and warmed so that it reached room temperature (about 23° C.) in twenty minutes. The experiments reported below were conducted with propyl alcohol.

*Determination of propyl alcohol after warming solution to room temperature.*

Propyl alcohol used.	Perman-ganate consumed.	Amount found.	Per cent found.
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	
0.1215	0.2490	0.1183	97.4
.1458	.3050	.1449	99.4
.1701	.3600	.1710	100.5
.1215	.2495	.1185	97.4
.1215	.2530	.1202	98.9
.1458	.3040	.1444	99.0



That it should be necessary to warm the solution to room temperature seems inconsistent with the results obtained on propyl and isobutyl alcohols where the acids formed were distilled. The explanation probably lies in the fact that delay occurred before the destruction of the permanganate with the hydrogen peroxid. Hence it seems a necessary condition in the quantitative oxidation of mixtures containing isobutyl and amyl alcohols by alkaline permanganate, that after the oxidation of the amyl alcohol at 0° the solution be allowed to warm to room temperature.

That amyl alcohol in carbon tetrachlorid solution should be quantitatively oxidized by alkaline permanganate was quite probable, providing the alkaline permanganate could reach the alcohol. Knowing that the oxidation of the amyl alcohol is quite rapid, and desiring to shorten the time of shaking as much as possible, it was thought five minutes of continual shaking would be sufficient. The following table indicates the amount of amyl alcohol dissolved in 100 cc of carbon tetrachlorid and the amount found:

*Determination of amyl alcohol in carbon tetrachlorid solution.*

Amyl alcohol used.	Amount found.	Per cent found.
<i>Gram.</i>	<i>Gram.</i>	
0.1004	0.1029	102.4
.1671	.1704	101.9
.1892	.1931	102.1
.2053	.2064	100.5
.2450	.2467	100.7

The extraction of amyl alcohol from alcoholic solution was next subjected to experiment. In the analysis of distilled liquors we are dealing with 45 to 55 per cent by volume alcohol solutions which are extracted with tetrachlorid and the tetrachlorid washed with saturated solutions of sodium chlorid and sodium sulphate to remove the ethyl alcohol, estimating the higher alcohols extracted under fixed conditions. It was thought of importance to learn how thoroughly the washing of the ethyl alcohol from the tetrachlorid was accomplished by the customary method of washing three times with three 50 cc portions of saturated sodium chlorid solution, and lastly with one 50 cc portion of saturated sodium sulphate solution. The extraction was made from 100 cc of 45 per cent by volume alcohol. The temperature of the solutions was 23° C. In four experiments the following amounts (grams) of permanganate were consumed: 0.0080, 0.0114, 0.0090, 0.0065; average, 0.0087. This indicated the retention in the carbon tetrachlorid of approximately 0.0033 gram of ethyl alcohol.

Extraction of known amounts of amyl alcohol from ethyl alcoholic solution, 45 per cent by volume, were made with the following results:

*Extraction of known amounts of amyl alcohol from ethyl alcohol solution (25° C.).*

Amyl alcohol present.	Total permanganate consumed.	Correction for ethyl alcohol.	Permanganate consumed by amyl alcohol.	Amyl alcohol found.	Extraction.
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	<i>Per cent.</i>
0.0987	0.1055	0.0087	0.0968	0.0673	68.2
.1480	.1668	.0087	.1581	.1100	74.3
.2468	.2725	.0087	.2638	.1826	74.0

*Extraction of similar known amounts of amyl alcohol at 17.5° C., using 100 cc of a 50 per cent by volume solution of ethyl alcohol.*

Amyl alcohol used.	Total permanganate consumed.	Correction for ethyl alcohol.	Permanganate consumed by amyl alcohol	Amyl alcohol found.	Extraction.
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	<i>Gram.-%</i>	<i>Gram.</i>	<i>Per cent.</i>
0.0987	0.1205	0.0087	0.1118	0.0778	78.8
.1481	.1810	.0087	.1723	.1199	81.0
.1481	.1730	.0087	.1643	.1143	77.2
.1974	.2295	.0087	.2208	.1537	77.9

For the estimation of ethyl alcohol by alkaline permanganate a solution of fusel-free alcohol containing 24.75 grams per 100 cc as determined by the pycnometer was diluted with water one hundred times. Portions of this solution were then diluted to 50 cc and estimated by the same method as used in the case of the other alcohols. The oxidation was quite rapid, and the time allowed was ten minutes. Using the factor 0.3636 for oxidation to acetic acid the following results were obtained:

*Estimation of ethyl alcohol by alkaline permanganate.*

Permanganate used.	Ethyl alcohol.		
	Amount present.	Amount found.	Per cent found.
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	
0.1392	0.0495	0.0506	102.2
.1673	.0619	.0608	98.2
.2072	.0742	.0753	101.4
.2045	.0742	.0743	100.1
.2070	.0742	.0753	101.4
.2812	.0990	.1021	103.1
.3405	.1237	.1237	100.0

Similar trials were made with methyl alcohol. Methyl alcohol was purified by drying with calcium chlorid, heating with anhydrous oxalic acid to convert into dimethyl oxalate, the crystals drained and pressed dry, and finally decomposed with slight excess of dilute alkali and distilled. The specific gravity of the distillate was taken and the methyl alcohol present obtained from Dittmar's table. In the first series of experiments the oxidation was allowed twenty minutes at the temperature of the ice bath. Considering formic acid to be the end product of the oxidation, the factor would be 0.253; if the formic acid were completely oxidized to carbon dioxide the factor would be 0.1686.

*Estimation of methyl alcohol by alkaline permanganate at 0° C.*

Methyl alcohol present.	Permanganate consumed.	Methyl alcohol found.			
		Factor 0.253.		Factor 0.1686.	
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	<i>Per cent.</i>	<i>Gram.</i>	<i>Per cent.</i>
0.0196	0.1145	0.0289	147.5	0.0193	98.5
.0392	.2150	.0544	143.9	.0362	92.4
.0589	.3130	.0791	135.0	.0527	89.5
.0981	.4115	.1041	106.1	.0693	70.8

It will be inferred that the reaction proceeded to carbon dioxide, but was not complete when large amounts of methyl alcohol were oxidized. There is no need of cooling the solutions if complete oxidation is intended, so a series of trials were made at room temperature, time of oxidation thirty minutes, and using the factor 0.1686. The results follow:

*Estimation of methyl alcohol by alkaline permanganate at room temperature.*

Methyl alcohol present.	Permanganate consumed.	Methyl alcohol found.	Per cent found.
<i>Gram.</i>	<i>Gram.</i>	<i>Gram.</i>	
0.0196	0.1160	0.0196	100.0
.0392	.2300	.0388	99.0
.0392	.2305	.0389	99.2
.0587	.3380	.0570	97.1

When warmed in a water bath at 60° for five minutes and cooled to room temperature and titrated, 0.0393 gram of methyl alcohol gave 0.0395 or 100.8 per cent of the amount present.

For the application of the alkaline permanganate method to the carbon tetrachlorid extract of the higher alcohols from distilled liquors, the following procedure is suggested:

SOLUTIONS REQUIRED.

1. A stronger solution of potassium permanganate containing approximately 20 grams to the liter.
2. A hydrogen peroxid solution of a strength slightly in excess of that of solution No. 1 (2 per cent stronger).
3. A standard permanganate solution containing 10 grams of the salt to the liter, the value of which has been accurately ascertained.
4. A solution of potassium hydroxid of 1 : 1 strength.
5. A sulphuric-acid solution containing approximately 25 per cent of acid.

METHOD OF PROCEDURE.

To the carbon tetrachlorid extract contained in the separatory funnel, add 10 cc of the potassium hydroxid solution (No. 1). Cool the mixture in ice water to approximately 0° C. Similarly cool 100 cc of the stronger solution of potassium permanganate (No. 1), accurately measured, in a flask.

To the contents of the separatory funnel add the bulk of the permanganate solution, but without rinsing the flask and retaining the residue to be added at a later stage.

Remove the mixture from the bath and shake vigorously for a period of five minutes; set aside for thirty minutes, with occasional shaking, permitting the liquid to warm to room temperature (20° to 25° C.).

Accurately measure 100 cc of hydrogen peroxid solution into a 1-liter Erlenmeyer flask. Acidulate this with 100 cc of sulphuric-acid solution. Slowly add the contents of the separatory funnel with constant shaking, keeping the acid solution constantly in excess.

Rinse the separatory funnel and flask containing the residue of permanganate with water and add to the peroxid solution.

Titrate the excess of hydrogen peroxid remaining with the standard potassium permanganate.

Run a blank determination, using the same amounts of the stronger permanganate, potassium hydrate, hydrogen peroxid, and sulphuric-acid solution and titrating the residual peroxid with the standard permanganate as before.

The difference in amount of permanganate consumed in grams times 0.696 gives the result in terms of amyl alcohol.

## METHODS OF ANALYSIS OF DISTILLED SPIRITS.

By L. M. TOLMAN and W. E. HILLYER.

The methods of the association for the analysis of distilled spirits, as given in Bulletin 107, Revised, of the Bureau of Chemistry, are for the most part the best methods available; but a few modifications and some new methods have been found to be of value.

## DETERMINATION OF COLORING MATTERS.

The method which has proved to be the most satisfactory in the Bureau of Chemistry for distinguishing between natural and artificial coloring matters in distilled spirits is the qualitative Marsh test. This depends on the relative solubilities of coloring matters in ethyl alcohol, amyl alcohol, and water. The addition of amyl alcohol, when in sufficient quantity, to a mixture of 50 parts of ethyl alcohol and 50 parts of water will cause a separation of the liquids into two layers, the lower layer being largely water and the upper one a mixture of ethyl alcohol, amyl alcohol, and some water. As a result of this separation, water-soluble coloring matter can be separated from alcohol-soluble coloring matter; that is to say, caramel can be separated from the natural coloring matter of whisky. Up to the present time this has been used as a qualitative test of the greatest value, but it now appears that the method can be adapted for quantitative determination, the amount of added coloring matter present in relation to the natural coloring matter being determined. The following method has been developed and found to be entirely satisfactory:

## AMYL INSOLUBLE METHOD (QUANTITATIVE MARSH TEST).

Evaporate 50 cc of the whisky just to dryness on the steam bath in a porcelain evaporating dish. Add 26.3 cc of 95 per cent alcohol to dissolve the residue, and transfer to a 50 cc flask, using water and making up to volume with water. This gives a 50 per cent alcoholic solution from which to make an extraction. [It is necessary that the extraction should be made from a solution of definite alcoholic strength, as it can be readily seen that variations in the percentage of ethyl alcohol would make a decided difference in the amount of amyl alcohol to effect the proper separation.] Place 25 cc of this 50 per cent alcoholic solution in a separatory funnel, add 20 cc of the Marsh reagent, then shake lightly so as not to emulsify. (The Marsh reagent consists of 100 cc of amyl alcohol, 3 cc of sirupy phosphoric acid, and 3 cc of water; shake before using.) Allow the layers to separate; repeat this shaking and standing twice more, and after the layers have clearly separated the last time, draw off the lower or water layer containing the caramel or water-soluble coloring matter into a 25 cc cylinder and make up to volume with 50 per cent alcohol. Compare this portion in a colorimeter with the remaining 25 cc which has not been treated with the Marsh reagent, thus directly giving the percentage of color not soluble in amyl alcohol.

The following table gives the results obtained by applying this method to straight and imitation whiskies:

*Amyl alcohol tests for color in whiskies.*

Description of sample.	Insoluble in amyl alcohol.	Soluble in amyl alcohol.	Description of sample.	Insoluble in amyl alcohol.	Soluble in amyl alcohol.
Straight American whiskies:	<i>Per cent.</i>	<i>Per cent.</i>	Imitation whiskies bought in market:	<i>Per cent.</i>	<i>Per cent.</i>
Average.....	10	90	Average.....	86	14
Maximum.....	12	88	Scotch whiskies (straight):		
Minimum.....	7	93	Average.....	7	93
Straight whiskies bought in market:			Maximum.....	8	92
Average.....	9	91	Minimum.....	5	95

It will be seen from this table that in any straight American whisky 90 per cent of the coloring matter is soluble in the amyl alcohol and ethyl alcohol layer, while in an imitation whisky 14 per cent is soluble in the upper layer. This method gives a much more complete separation of the coloring matter taken from wood and from caramel than either the water-insoluble method or the ether-soluble method, and seems to be the most reliable and satisfactory test that we now have for the detection of added coloring matter and its estimation.

Further, if a whisky contains a certain amount of caramel this method will give a partial separation, and the percentage of caramel added can be approximately estimated. The present provisional method, known as the "Crampton and Simons test," for caramel depending on the insolubility of caramel in ether, is not nearly so satisfactory as the method here presented, as the separation of the coloring matters is much less complete. The ether-soluble method, as given in Bulletin 107, Revised, page 101, is cumbersome, and calls for unnecessary special apparatus. Accurate practical results have always been obtained by the following procedure:

#### ETHER-SOLUBLE COLOR METHOD.

Evaporate 50 cc of the sample just to dryness on the water bath; wash into a 50 cc flask with 25 cc of alcohol and dilute to mark with water. Transfer 25 cc with a pipette to a separatory funnel and add 50 cc of ether. Shake at intervals for half an hour, let settle, draw off the aqueous layer, and make up to 25 cc with water. Mix this latter and compare with the 25 cc of the solution which were not treated with ether. Express the amount of color removed on the percentage basis as ether-soluble color.

This modification simply eliminates the special Bromwell apparatus, and the method is but little used in the Bureau of Chemistry, but the change is presented as essential in applying the method. We do, however, use the method of determining the color insoluble in water, caramel, of course, being perfectly soluble and the coloring matter of whisky being practically insoluble in water. This gives a method of separation which is very satisfactory, the procedure outlined in this laboratory being as follows:

#### WATER-INSOLUBLE COLOR METHOD.

Evaporate 50 cc of sample just to dryness. Take up with cold water, using approximately 15 cc, and filter, washing with water until nearly 25 cc of filtrate is obtained. Add about 26.3 cc of 95 per cent alcohol, and complete the volume to the 50 cc mark by the addition of water. Mix thoroughly and compare in a colorimeter with the color of the original sample, stating results as percentage of color insoluble in water obtained by subtracting the percentage soluble color, reading from 100.

The following table compares the results obtained by the determination of water-insoluble color with the ether-soluble color on a number of straight whiskies and on spirits colored with caramel:

*Comparison of water-insoluble color and ether-soluble color on different types of distilled spirits.*

Description of samples.	Water-insoluble coloring matter.	Ether-soluble coloring matter.
	<i>Per cent.</i>	<i>Per cent.</i>
69 straight rye whiskies of known source and age.....	71.0	35.0
27 straight bourbon whiskies of known source and age.....	68.1	29.5
24 compound whiskies bought in open market.....	12.2	19.3
33 imitation whiskies bought in open market.....	6.7	7.3

This table shows that results obtained by the ether-soluble method do not show enough difference between the straight whiskies and those artificially colored. That is to say, in ether the difference in solubility between whisky color and caramel is

not large enough to make a satisfactory separation, while the water-insoluble method shows a much wider difference and gives the same information, but is not so valuable as the amyl alcohol test, which makes the most complete separation of the two kinds of coloring matter. In a study of the water-insoluble method it was found best to evaporate just to dryness, and, further, that the manner of evaporation did not affect the results. It also appears that the amount of sugar present as caramel does not affect the solubility of the whisky coloring matter.

#### DETERMINATION OF FUSEL OIL.

The determination of fusel oil or higher alcohol is undoubtedly one of the most important ones made in the analysis of distilled spirits, giving more information as to the methods of distillation in the manufacture of the spirit than any other factor. When the examination of distilled spirits was begun, an extensive investigation was made of the Roesse method as given in the official methods of the Association of Official Agricultural Chemists,<sup>a</sup> and it was found that a great many difficulties were encountered in employing the apparatus and method as there directed. This method, depending as it does upon the relative solubilities of alcohol, chloroform, and fusel oil in each other, requires that the conditions of temperature and concentration must be very carefully controlled. The first difficulty encountered was the leaking of the stopcocks in the apparatus adopted by the association, known as the Bromwell tube, and after many experiments it appeared that this could not be overcome. The chloroform solution would invariably leak through the ground-glass stoppers, so that it became necessary to return to the older form of apparatus as designed by Roesse, which has no stopcock, but is extremely difficult to fill. With this form of apparatus, however, somewhat satisfactory results were obtained.

It was found also to be absolutely necessary that the apparatus be perfectly clean and free from any oily material, and in order to insure this it was heated in a sulphuric-acid-bichromate solution after almost every determination: Unless this is done drops of water will stick to the sides of the chloroform bulb and increase the volume of the chloroform and the amount of fusel oil shown. Also it is absolutely necessary that during the whole determination the solutions and apparatus should be kept exactly at 15° C., and to this end a large constant temperature bath was built deep enough so that the tubes could be immersed completely and the shaking could be carried on in the bath itself. It was found that if the tubes were removed from the bath and shaken in the air the results were entirely inaccurate, a much larger blank being obtained. This is easily explainable. If the temperature of the room is very much above that of the bath, the shaking will raise the temperature of the solution and change the relations between the solubilities of the various liquids in each other, thus yielding results of little value.

A regular procedure was adopted in regard to the shaking. The apparatus was filled according to directions and immersed in the constant-temperature bath until all the solutions had reached the same temperature. The tube was inverted in the bath and shaken vigorously 150 times, then reversed and allowed to stand in the tank until all the chloroform had settled back into the bulb, after which a reading was made.

By using this apparatus and carefully following these details fairly satisfactory results were obtained, but at the same time the Allen-Marquardt method was tested and found to be much more convenient and accurate. In a recent paper by Doctor Dudley, of Vanderbilt University, on "The comparison of results obtained by the Roesse and the Allen-Marquardt methods,"<sup>b</sup> these same difficulties and errors in the Roesse method were noted, so that it seems advisable to abandon the old Roesse method and direct our attention to the Allen-Marquardt method, which apparently gives much more satisfactory results.

<sup>a</sup>Bul. 107, Revised, p. 97.

<sup>b</sup>J. Amer. Chem. Soc., 1908, 30:1271.

In the work of the Bureau of Chemistry for the past year or more much work has been done to perfect the Allen-Marquardt method, and it will be discussed somewhat in detail, as some of the modifications devised improve the method and have not been published, but are of great importance in obtaining accurate results.

The method as used at the present time is the same as is given in Bulletin 107, Revised, but it has been found necessary to have the proof of the sample under examination not much above 100 in order that the volume when made up to 1.12 specific gravity will not be too great for the separatory funnels used. In the analysis of high-proof spirits, therefore, 50 cc of the sample are used for analysis and 50 cc of water added, making the product approximately 100 proof.

One point which is extremely important is that the carbon tetrachlorid used must be of the highest purity. We have found that most of the C. P. carbon tetrachlorid on the market is entirely unsatisfactory for this determination until it has been purified by oxidation with bichromate and sulphuric acid, as called for in the present provisional method. In the proper control of this purification a renewal of the bichromate and sulphuric acid mixture is necessary and often makes the process a lengthy one where the carbon tetrachlorid is very impure. For this reason the following new method, devised by A. M. Breckler, is used:

Mix the crude carbon tetrachlorid with strong sulphuric acid in the proportion of 300 cc of acid to every 3,000 cc of the carbon tetrachlorid. Shake this mixture thoroughly at frequent intervals and allow to stand over night. Then run water through the mixture continuously, by means of a glass tube inserted to the bottom of the bottle and connect with the water tap, until thoroughly washed free from acid and impurities. Draw off the water or upper layer by means of a siphon, the last portions being removed as far as possible by a pipette. Add an excess of soda solution and distil the carbon tetrachlorid from it.

The advantage of this method is that a good blank can be obtained, the process of purification is decidedly shorter, and it may be adapted to cruder carbon tetrachlorid than can the present provisional method, thus allowing cheaper material to be used. A blank should always be run on each set of determinations and if this amounts in the end to more than 0.2 to 0.3 cc of tenth-normal alkali due to the carbon tetrachlorid, the reagent is not pure enough for this determination. The impurities present in some samples of bichromate also gave trouble. It is absolutely necessary in this method that reagents of all kinds shall be entirely free from organic contamination.

In the extraction portion of the method the following precautions are necessary:

(1) A shaking machine gives more regular conditions for extraction, each shaking process being continued for a period of two minutes.

(2) It is of advantage to have perfectly saturated sodium chlorid and sodium sulphate, and for this purpose the solutions are kept standing over an excess of the salt and continually agitated by a current of air.

(3) It has been experimentally determined that a colder temperature gives a more efficient extraction.

(4) It is also necessary to take special care to remove by complete washing with sodium sulphate all the sodium chlorid from the carbon tetrachlorid extract on account of the formation of chlorin in the oxidizing process with bichromate and sulphuric acid and the danger of this chlorin interfering with the titration by bleaching the indicator. The present provisional method calls for one washing with sodium sulphate to accomplish this result, but it has been found by experiment that two are not sufficient to remove the sodium chlorid. On the other hand, it may be that more than two washings would abstract some of the higher alcohols, therefore it appears that the present directions in the provisional method should be changed from one to two washings.

(5) It is necessary in carrying on the oxidation that the boiling of the carbon tetrachlorid with the oxidizing solution should be slow and regular, and that a high condenser should be used to insure the complete condensation of all the products. Especially is this slow boiling necessary in the following modified method which depends on the estimation of the potassium bichromate reduced during the oxidation.

The Allen-Marquardt method, modified according to these suggestions, reads as follows:

MODIFIED ALLEN-MARQUARDT METHOD.

*Reagents.*

*Solutions of sodium thiosulphate.*—Two solutions of sodium thiosulphate are used, one an approximate three-fourths-normal not standardized, and the other a tenth-normal standardized against pure potassium bichromate whose value has been obtained against pure iron.

*Carbon tetrachlorid.*—The purification of this reagent is a fundamental necessity. (See Breckler's method, p. 209.)

*Potassium iodid solution.*—Dissolve 1 gram in every cubic centimeter of water taken.

*Bichromate oxidizing solution.*—Dissolve 200 grams of pulverized potassium bichromate in 1,800 cc of water and add 200 cc of concentrated sulphuric acid.

*Determination.*

Proceed with the Allen-Marquardt method for determining fusel oil, as given in Bulletin 107, page 98, to the point of adding the oxidizing mixture. Add exactly 50 cc of the oxidizing solution to the blank and the samples by means of a pipette or burette and then oxidize under a high reflux condenser for eight hours. During the oxidation, shaking the flask with a rotary motion will prevent any isolation of spots of bichromate on the flask below the carbon tetrachlorid. Decomposition from overheating is prevented by placing between the wire gauze and the flask two thicknesses of one-fourth inch asbestos board.

Remove the flask from the reflux condenser and separate the bichromate from the carbon tetrachlorid in a separating funnel. Care must be taken that in this process no bichromate is lost and that the carbon tetrachlorid is washed free from it. Make up the bichromate solution thus obtained to 500 cc.

Measure 200 cc of this solution into a liter flask. Add 50 cc of the potassium iodid solution, 50 cc of the approximately three-fourths-normal solution of sodium-thiosulphate, and then 20 cc of concentrated hydrochloric acid. Titrate the excess of bichromate with the standard tenth-normal thiosulphate solution. If a high content of fusel oil was present in the original sample, the addition of 50 cc of the three-fourths-normal thiosulphate solution may be excessive and if such is the case a smaller amount should be added and the blank titrated in the same manner.

Treat blanks containing exactly the same amount of the reagents used in running each series of commercial samples in the same way, starting them at the point where the carbon tetrachlorid is washed with sodium chlorid. The titration of this blank, to which has been added the same amount of the three-fourths-normal thiosulphate solution, gives the value of the oxidizing mixture. The difference between this value in cubic centimeters of tenth-normal thiosulphate and that obtained on the reduced oxidizing mixture of the commercial sample in each case gives the amount of bichromate used up by the oxidation of the fusel oil present. This difference is then calculated to grams of amyl alcohol using the following factor: 1 cc of tenth-normal thiosulphate equals 0.001773 gram of amyl alcohol.

The factor used is an average one obtained by three manipulators in making 60 runs on standards containing amounts of pure amyl alcohol, varying from 0.05 to 0.5 gram as follows:

*Development of factor in the oxidizing process of the Allen-Marquardt method.*

Analyst.	Content of amyl alcohol.	Number of determinations.	Maximum.	Minimum.	Average.
	<i>Gram.</i>				
Boyle .....	0.05+	9	0.001885	0.001652	0.001751
Do. ....	.10+	8	.001885	.001637	.001785
Albrech. ....	.10—	5	.001847	.001806	.001806
Palmore. ....	.10+	4	.001790	.001634	.001726
Boyle .....	.15+	9	.001824	.001721	.001776
Do. ....	.20+	8	.001840	.001762	.001799
Palmore. ....	.20+	5	.001751	.001710	.001710
Boyle .....	.31+	4	.001754	.001719	.001736
Palmore. ....	.30+	5	.001725	.001819	.001771
Boyle .....	.42+	4	.001830	.001800	.001810
Do. ....	.53+	1	.001818	.....	.001818
.....	.....	.....	.001885	.001634	.001773



The maximum and minimum figures show that the oxidizing process carried out under normal laboratory conditions is practically uniform with respect to the varying amounts of fusel oil present. It was found that the reactions taking place between the bichromate and the amyl alcohol were little understood, and that it was impossible to calculate a factor which gave anything like the actual results obtained; so that the results of the experiments given above were made, and the surprising closeness of the figures obtained by the various analysts shows that there is a definite reaction taking place; and, while this reaction has not been figured out, the writers feel entirely justified by the results in adopting this factor.

This change in the method was developed as it was found that the final distillation of the volatile acids was not satisfactory, on account of the fact that only a portion is distilled over when the present provisional method is followed. The following table of experimental data develops the conclusion just stated and shows on an average for all contents of amyl alcohol that the percentage yield is raised from 78 to 92 per cent if the washing process is continued.

*Effect of continued washing on the results obtained by the Allen-Marquardt method.*

Amyl alcohol present.	Present provisional method.		Amounts of amyl alcohol recovered by additional washings (expressed as cc tenth-normal alkali).				Total yield by additional washing.	
	Amounts obtained.	Percentage obtained.	First.	Second.	Third.	Fourth.	Amount.	Per cent.
<i>Gram.</i>	<i>Gram.</i>						<i>Gram.</i>	
0.05.....	0.034 .037 .035 .038 .049 .067	72.0						
0.100.....	.073 .067 .066 .108	68.0						
0.150.....	.107 .111 .119	71.0						
0.200.....	.128 .123 .136 .150	63.5						
0.250.....	.146 .162 .145 .179	60.0						
0.300.....	.190 .184 .175	60.7						
0.100.....	.085	85.0	0.7	0.2	0.3	0.5	0.096	96
0.100.....	.089	89.0	.9	.3	.0	.0	.096	96
0.100.....	.096	96.0	.3	.3	.2	.0	.103	103
0.100.....	.087	87.0	.8	.3	.2	.0	.096	96
0.100.....	.090	90.0	.2	.3	.3	.1	.098	98
0.100.....	.087	87.0	.5	.4	.1	.1	.097	97
0.100.....	.074	74.0	.8	.6	.8	.4	.097	97
0.100.....	.070	70.0	2.1	.9	.3	.0	.099	99
0.200.....	.190	95.0	.5	.1	.1	.0	.196	98
0.300.....	.237	79.0	.5	.2	.2	.1	.246	82
0.300.....	.239	80.0	.4	.4	.2	.2	.250	83
0.300.....	.238	79.0	.4	.1	.2	.2	.246	82
0.300.....	.225	75.0	.8	.3	.2	.3	.245	80
0.350.....	.294	84.0	1.1	.6	.3	.2	.313	89
0.350.....	.286	81.0	.4	.8	.2	.2	.303	87
0.350.....	.272	78.0	.5	.3	.2	.3	.283	81
Average.....		78.0						92

\* By the fifth washing 0.3 cc were obtained.

The curves plotted in fig. 5 show the relationship between the percentage yields of the present provisional method, of the impractical prolonged washing method, and of the proposed modified Allen-Marquardt method. Only the figures obtained in testing the portion of the Allen-Marquardt method which follows the beginning of the oxidation process are represented.

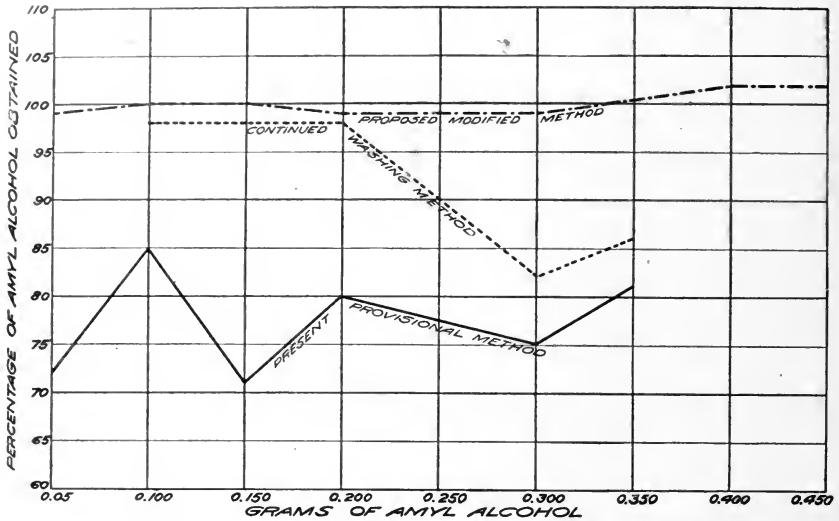


FIG. 5.—Comparison of three methods for the determination of amyl alcohol.

The curve shows that uniform results can not be obtained by the present provisional method, and that if the washing and distillation method, an impractical procedure, be adopted, high yields will probably only be obtained on the low-content samples. The curve developed by the runs on varying amounts of amyl alcohol by the proposed modification of the Allen-Marquardt method gives a higher and more uniform yield for all amounts and one which approximates closely to 100 per cent. The work of developing this latter curve represents 62 runs on varying amounts of amyl alcohol from 0.05 to 0.53 gram, and the manipulation of three analysts.

The fact of obtaining a higher and more uniform yield caused us to prepare and send out to 18 collaborators, 11 of whom reported, the samples described in the report of the associate referee on distilled spirits for this year (see p. 25). The comparison of the old and the modified method and the results of the collaborative work are there presented by the table and curves, and distinctly show the advantages of the proposed modified method.

DETERMINATION OF THE IODIN NUMBER OF THE NONVOLATILE  
ETHER EXTRACT OF PAPRIKA.

By W. DENIS.

As in this laboratory much difficulty was experienced in obtaining concordant results on determinations of the iodine number of the nonvolatile ether extract of paprika, 8 samples of ground paprika sent in as suspected of being adulterated with olive oil, and 1 sample composed of the shells of Hungarian paprika ground in the laboratory were examined, various methods of extraction being used.

*Method 1.*—This consisted in digesting 10 grams of paprika over night with 100 cc of ordinary ether in a stoppered flask. The next morning all ether was decanted off through a double filter and the residue thoroughly washed with 200 cc more ether. The ether was then distilled off and the residue dried to constant weight at 100° C. and calculated as nonvolatile ether extract, the iodine number of the same being determined on portions of this residue by the official method, using the Hanus solution.

*Method 2.*—Manipulation the same as in method 1 except that petroleum ether B. P. 50–60° was substituted for sulphuric ether.

*Method 3.*—The official method for the determination of nonvolatile ether extract in spices as given in Bulletin 107.

*Method 4.*—The Doolittle-Ogden method of extraction with cold anhydrous ether.<sup>a</sup>

All four of the above methods proved unsatisfactory. Methods 1 and 2 were at once discarded because it was found after repeated trials to be absolutely impossible by these methods to obtain portions of identical composition from one lot of ether extract, due to the fact that on standing for a few minutes at 100° in the water oven several drops of a colorless oil appeared on the sides of the container, while on standing at room temperature needle-shaped crystals, which on microscopic examination proved to be crystals of fat, were seen in the deep red residue. It was also frequently found that in spite of careful filtration minute quantities of some body difficultly soluble in chloroform were present in the extract, thus further interfering with the accuracy of the results.

By the official method also it was found difficult to obtain duplicate results, apparently due to the fact that a drying oil is present in the extract which may oxidize to varying degrees depending on slight differences in manipulation during the long period of extraction. By the Doolittle-Ogden method some difficulty, although not so much as with the three preceding methods, was experienced in obtaining good duplicate determinations of the iodine number; and in addition this method has the disadvantage of allowing only one determination of the iodine number to be made on the product of a single extraction. The following modification of the Doolittle-Ogden method was finally devised, and so far has given satisfactory results, giving duplicates on iodine number determinations agreeing to within 0.2 per cent.

*Method No. 5.*—Ten grams of paprika spread in a thin layer on a flat-bottomed dish are dried for two hours in a vacuum oven at 60° and 25 mm. The material is then transferred to a double filter and washed with 300 cc of cold anhydrous alcohol-free ether. After distilling off the ether the residue is taken up with fresh ether and filtered into a small tared beaker, the filter paper being carefully washed with ether to remove all trace of oil. After again evaporating off the ether the residue is dried to constant weight at 100°. After the final weighing the residue is washed with chloroform into a 100 cc flask and made up to volume with this liquid. Determinations of the iodine number are made on 10 cc portions of this solution, thus making possible several duplicate determinations on the residue obtained in a sample extraction.

<sup>a</sup>J. Amer. Chem. Soc. 1908, 30: 1481.

The following points are noted as having been brought out by the limited data obtained:

(1) Any method in which portions of the extract are poured off into shell vials, etc., for the determination of the iodine number, as is customary in the determination of this constant with oils and fats, should be avoided, for as before stated it is absolutely impossible by this method to obtain two portions of identical composition.

(2) Iodine numbers determined on the nonvolatile ether extract of paprika, which by the high value of this constant would appear not to be adulterated with olive oil, are found when made on the product obtained by the official method of extraction to be considerably lower than those obtained on the product produced by extraction with cold solvents, indicating perhaps the presence of drying oils; while, as is to be expected, the percentage of nonvolatile ether extract is lower. On the other hand, with commercial paprikas adulterated with olive oil the difference between the iodine numbers obtained by the two methods of extraction is not so marked. Extraction with cold petroleum ether, boiling point 50°-60° gives a nonvolatile extract about 1 per cent lower than is obtained by the use of an equal volume of sulphuric ether under identical conditions. In the following table samples Nos. 1 to 8 are commercial paprikas sent in on suspicion of adulteration with olive oil while No. 9 is a pure product prepared by grinding the shells of Hungarian paprika.

*Comparison of methods for determination of iodine number and nonvolatile ether extract.*

No.	Iodine number.		Nonvolatile ether extract.			
	Official method.	Method No. 5.	Official method.	Method No. 5.	Method No. 1.	Method No. 2.
			<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1	127.1	139.0	15.8	9.64	11.99	11.07
2	121.6	127.3	17.5	12.29	13.84	12.98
3	108.0	113.6	21.3	15.14	16.76	-----
4	124.4	130.2	15.8	11.52	13.21	12.57
5	107.7	113.3	21.75	17.00	18.73	17.97
6	109.0	114.5	20.1	15.74	17.38	16.54
7	107.3	114.4	20.6	15.63	17.28	16.36
8	-----	130.3	-----	13.66	-----	-----
9	127.0	139.0	5.04	2.84	-----	-----

## DETERMINATION OF STARCH IN COCOA PRODUCTS.

By W. L. DUBOIS.

The provisional method for the determination of starch in cocoa and cocoa products requires grinding of the sample in a mortar repeatedly with ether and pouring the solution through filter paper each time until the fat is extracted. With sweetened material the fat-free residue is then rubbed in a mortar to a paste with water and filtered on the same paper, the process being repeated until all the sugar is removed, which requires about 500 cc of water. This process is a very slow and tedious one. The manipulation of the sample in the mortar with ether both in the grinding and subsequent pouring requires extreme care to prevent loss. The filtration in many cases is very slow and with sweetened samples it often takes two days to wash with 500 cc of water. In order to overcome this objection the following procedure was tried: Four grams of the unsweetened sample or 8 grams of the sweetened goods are shaken with 100 cc of gasoline in an ordinary 8-ounce, short neck, nursing bottle until the material is completely disintegrated; the bottle is whirled in a centrifuge until the supernatant liquid is clear and the gasoline drawn off with a small tube attached to vacuum pump and the process repeated. This procedure removes practically all the

fat and prepares the sample for the next operation. In case of unsweetened material this merely consists in washing the same into a 500 cc Erlenmeyer flask with 200 cc of water and proceeding with the hydrolizing and determination of starch as directed in the provisional method. With the sweetened goods after the extraction of fat with gasoline 100 cc of water are added to the residue and the bottle shaken thoroughly and whirled in the centrifugal machine. If the speed of the machine be sufficiently high a clear water solution may be obtained, although as a rule a thin layer of chocolate will float on the top. A small pipette may be passed through this layer into the water solution and the same withdrawn from the bottom. Where such high speed can not be obtained, however, it is necessary to pass the water solution through filter paper to remove the suspended particles. The process is repeated and the residue transferred to the filter paper and washed with sufficient water to make a filtrate of 500 cc. This process requires a very much shorter time than that outlined by the provisional method.

From the table it will be seen that the extraction of fat and sugar is apparently complete, the results where comparisons were made with the provisional method being slightly higher than those obtained by that method, duplicates agreeing fairly well.

*Comparison of methods for the determination of starch in cocoa products.*

Sample.	Modified method.	Provisional method.	Sample.	Modified method.	Provisional method.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>
Cocoa nibs.....	{ 13.05 12.68 }	{ 10.77 }	Bitter chocolate.....	{ 13.33 12.79 }	{ 12.42 12.32 }
Bitter chocolate.....	{ 11.81 12.41 }	{ 11.38 }	Sweet chocolate.....	{ 8.07 8.22 }	{ 7.42 7.69 }
Bitter chocolate.....	{ 13.15 13.64 }	{ 12.50 12.90 }	Sweet chocolate.....	{ 8.51 Lost. }	{ 7.80 7.40 }

## MONDAY—AFTERNOON SESSION.

### EXAMINATION OF OYSTERS.

By W. D. BIGELOW.

There has long been a practice among those shipping oysters in the shell to place them for a day or two in a stream or fresh or brackish water. This process is commercially termed "drinking," and is practiced for the purpose of plumping the oysters. It is stated that at the beginning of the ebb tide the oysters open their shells and "drink." What really happens is that the fresher water diffuses into the oysters by osmosis and gives them a fictitious appearance of plumpness.

This practice of "drinking" oysters in the shell has been largely discontinued. Practically the same thing is accomplished, however, by soaking them for a considerable time in fresh water after their removal from the shell. As the purity of the water can be better controlled by this means, it is to be preferred to the older process of "drinking" the oyster before shucking. In either case, the plumping of the oysters is stated by shippers to be for the purpose of improving the product. This improvement, however, is entirely fictitious, the increased plumpness being due merely to the addition of water which is given off on cooking.

It is believed that the unnecessary addition of water to oysters, either directly or by means of ice, is objectionable on two grounds: First, it produces a fictitious appearance of plumpness; and, second, the weight of the oysters is increased by a substance

which does not add to their nutritive value; that is, a substance (water) is mixed with them in such a manner as to reduce their quality or strength.

When the oyster is removed from the bed by dredging or tonging, the inside of the shell contains a considerable amount of dirt and sand. In order to remove this the oysters are sometimes placed on floats at some convenient place in the salt water. Here, probably at the beginning of the ebb tide, they open their shells and "drink." Since the water in which they are placed is at the same concentration as that of the bed, however, there is no plumping or other change in their appearance except that during this process they appear to blow the dirt and sand from the shell and if the water be clean the oyster is fairly clean. They are then taken to the oyster house, shucked, and washed to remove the slime with which they are covered. It is said by shippers that this slime will rapidly produce decomposition and must be removed before the oysters are shipped. This washing, however, should not be prolonged more than is absolutely necessary for proper cleansing.

During the last season a study was made of the oysters in various parts of the country in order to secure data, if possible, by which oysters that had been properly treated might be distinguished from those which had been treated with an excessive amount of water. Seventy samples of oysters were taken from beds in various sections of the seacoast of the United States and sent to the laboratory without any treatment whatever. Other samples from the same beds were merely washed with water for a sufficient time to remove sand and dirt; while still other samples were soaked in water for a length of time varying from one hour to twenty-four hours.

When the samples arrived at the laboratory they were examined as follows: First, the total weight was taken, then the sample was strained through a colander and the solid meats and liquors weighed separately. Fifty grams of the oysters were then placed in a beaker with 200 cc of cold water and the whole heated in such a manner that the water was brought to the boiling point in about fifteen minutes. The boiling was continued for fifteen minutes, when the water was poured off as completely as possible, the oysters were cooled five minutes and weighed. From the figure thus obtained the per cent loss on boiling was determined. It was found that by continuing the boiling for a longer period than fifteen minutes the results were not greatly increased.

Another portion of the solid meats was passed through a meat chopper and the total solids, ash, and sodium chlorid determined in the usual way. The percentages of total solids and ash and sometimes of sodium chlorid were also determined in the liquor. From the figures obtained by the examination of the solid meats and liquors, together with their respective weights, the per cent of total solids in the original sample was calculated. In the majority of cases samples of the salt water were taken from the respective oyster beds and their content of sodium chlorid determined.

## SIMPLE TESTS FOR DETECTING BLEACHING IN FLOUR.

By A. L. WINTON and E. J. SHANLEY.

The Griess-Hosvay method for determining nitrites,<sup>a</sup> originally designed for water analysis, is generally recognized as the best means of detecting artificial bleaching in flour. Commercial unbleached flour contains no appreciable amount of nitrous acid, free or combined, while that bleached with nitrogen peroxid contains amounts increasing with the degree of bleaching. The quantitative process of determining nitrous acid, although not a tedious one, is, however, unnecessarily laborious when only qualitative results are desired. It involves the preparation of a standard nitrite solution and comparison of the intensity of the color produced in this solution with

<sup>a</sup> Sutton: Volumetric Analysis, 9th ed., 1904, p. 449.

that produced in the water extract of the sample in question, all of which can be dispensed with when the purpose is merely to learn whether or not the flour is bleached and whether the bleaching is light, moderate, or excessive. There has also been a demand not only for a simple and rapid method for detecting bleaching in the laboratory, but also for one which flour buyers, bakers, and consumers can carry out without special training and with simple apparatus.

The tests here described are, first, a simplification of the Griess-Ilosvay method and, second, a confirmatory test based on the observations of Alway<sup>a</sup> and others that the petroleum ether solution of unbleached flour is yellow, while that of bleached flour, if not excessively overbleached, is nearly colorless. The Griess-Ilosvay test is the more reliable, but the gasoline test, which depends on an entirely different principle, namely, the nature of the coloring matter of the fat, is useful for confirmation. A description of the tests in popular language follows:

#### I. GRIESS-ILOSVAY METHOD.

Place a heaping teaspoonful (10 grams) of the flour to be examined in a wide-mouthed, glass-stoppered 4-ounce bottle. Nearly fill with distilled water, or tap water free from an appreciable amount of nitrites, and add a teaspoonful (4 cc) of the test solution prepared as directed below, measured with a glass spoon. Cork the bottle and shake vigorously for a few minutes, then allow to settle for from fifteen to twenty minutes.

Under the above conditions bleached flour will impart to the liquid a color ranging from a light pink to a deep red, depending on the degree of bleaching. With unbleached flour the liquid is not colored a red tint, provided water free from nitrites is used. Always run, for comparison, a parallel test with a sample of unbleached flour, so that allowance can be made for any nitrites in the water.

*Test solution.*—1. Dissolve 0.5 gram of sulphanilic acid in 150 cc of dilute acetic acid (about 20 per cent). Keep well stoppered.

2. Dissolve 0.2 gram of alpha-naphthylamin hydrochlorid in 20 cc of strong acetic acid (glacial), and add 130 cc of dilute acetic acid (20 per cent). Keep well stoppered.

Mix 1 and 2 for use. The mixed reagent keeps for several weeks, and possibly much longer.

#### II. GASOLINE METHOD.

Place two heaping teaspoonfuls (20 grams) of the flour in a wide-mouthed, glass-stoppered 4-ounce bottle, add sufficient gasoline to nearly fill the bottle, shake, and allow to settle. If the flour is unbleached, the gasoline will become distinctly yellow; if bleached, it will remain nearly colorless. Conduct a parallel test on unbleached flour for comparison.

### A MODIFICATION OF THE BAMHIL TEST FOR DETECTING WHEAT FLOUR IN RYE FLOUR.

By A. L. WINTON.

This test depends on the presence of gluten in wheat flour and its absence in considerable amounts in rye and other flours. The original test, devised in 1852 by Bamihl,<sup>b</sup> a Prussian customs official, consists in rubbing up a small amount of flour with water on a microscopic slide by means of a cover glass and noting under the microscope whether or not gluten strings or rolls are formed. The objections to the test in its original form are that the microscope reveals the presence of traces of gluten in pure rye flour and under the microscope it is not possible to compare at a glance the amount found in pure rye flour with that from a suspected sample.

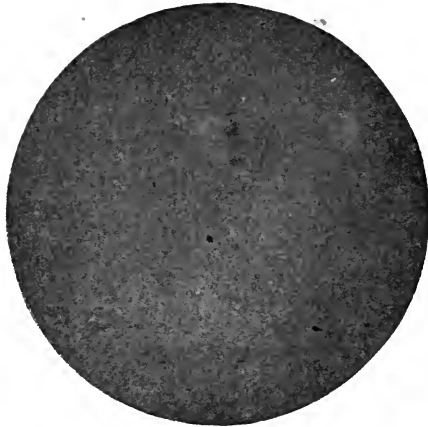
The writer's modification of the test consists in employing a dilute solution of eosin in place of water and dispensing with the microscope entirely. The gluten greedily

<sup>a</sup> Nebraska Agr. Exp. Sta., Bul. 102.

<sup>b</sup> Poggendorff, *Annalen Physik Chemie*, 1852, 85; 161.

absorbs the dye, and on a white background becomes very conspicuous because of its beautiful pink color. A description of the procedure follows:

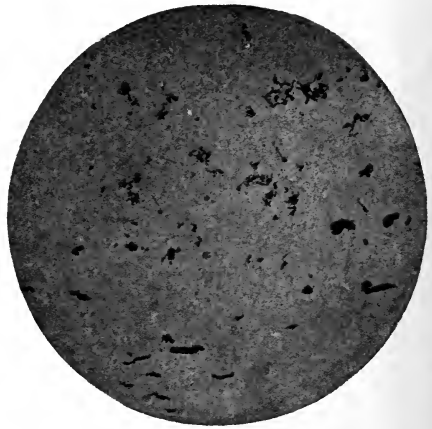
Place side by side on a microscopic slide 1.5 mg of the flour and a drop of water containing 0.2 gram of eosin in 1,000 cc. Allow the slide to rest on a sheet of white paper, and carefully mix the flour with the liquid by means of a cover glass, held between the thumb and finger in such a manner that it is raised slightly above the slide, taking care that none of the flour escapes from beneath it. Finally, allow the



1



2



3

FIG. 6.—Bamihl gluten test ( $\times 4$ ): 1, Pure rye flour showing only trace of gluten; 2, a mixture of 60 per cent rye and 40 per cent wheat; 3, pure wheat flour showing gluten masses.

cover glass to rest on the slide and rub it back and forth until the gluten, if present, forms into rolls or masses. Conduct parallel tests for comparison on pure wheat and pure rye flour. Proceeding in this manner, wheat flour yields an abundance of gluten, which is stained a beautiful pink color by the eosin, whereas rye flour yields none, or else only traces which are scarcely visible to the naked eye. Mixtures of rye and wheat flour yield variable quantities of gluten, depending upon the proportion of the two flours and their source.

In testing graham flour, buckwheat flour, and other cereal products containing considerable quantities of bran tissues or coarse lumps of any kind, the flour should be sifted through a bolting cloth before applying the test. The bolting is conveniently



carried out by placing a small quantity of flour in a beaker, covering the top with the bolting cloth held in place by means of a rubber band, inverting and shaking. Operating on the sifted material, as little as 5 per cent of wheat flour in buckwheat flour may be detected. In pure buckwheat flour I have never obtained a visible amount of gluten.

Figure 6, reproduced from photographs made by Mr. B. J. Howard, Chief of the Microchemical Laboratory, Bureau of Chemistry, shows the gluten obtained by this test in pure rye flour, a mixture of 60 per cent rye flour and 40 per cent wheat flour, and pure wheat flour, magnified 4 diameters. As has been stated, in practical work no magnification whatever is necessary to bring out the gluten strings or masses.

## MOISTURE DETERMINATIONS WITHOUT THE AID OF HEAT.

By P. F. TROWBRIDGE.

For more than a year at the Missouri experiment station the moisture determinations on meats have been made without the aid of heat, by drying in vacuum over sulphuric acid. At first the ordinary brass filter pump was used for obtaining a vacuum, aided by the use of about 10 cc of ether (Benedict method). A vacuum of 1 or 2 mm is easily obtained with good water pressure, but in warm weather many of the samples would show some putrefaction before desiccation was complete enough to check decomposition. It was noted that the water formed with the acid an upper layer, which generated considerable heat when mixed by rotation. This suggested the frequent agitation of the sulphuric acid in the bottom of the desiccators, with the result that twelve hours was sufficient to dry fresh meat samples so that they would not putrefy. Having considerable difficulty with the water pressure, a Geryk duplex vacuum pump was procured, and without the aid of ether a vacuum of less than 1 mm was secured in two or three minutes.

Substances such as brain and liver gave much trouble by frothing out of the moisture tubes as the air was being exhausted, but the difficulty was obviated by freezing these samples after they were weighed. Similarly, cold-water extracts of beef were frozen and evaporated to dryness in the vacuum without ever thawing, leaving the dry substance as a web-like mass the full size of the original frozen extract.

The moisture-free samples are used for determination of the ether-soluble material. Lean meats dry down to a very hard, horn-like mass, so that it is necessary to grind them and make a second extraction in order to obtain all of the ether-soluble material. In order to avoid this difficulty, the meat samples are mixed with ignited sand, and such good results are obtained that a description of the method may be of interest.

For the moisture and fat tubes use either the S. & S. extraction shells or the glass tubes with filter-paper bottoms. Fill the tube about one-third full of ignited sea sand and then stuff in a liberal amount of fat-free cotton. Dry the tubes thus prepared (they should be numbered) for several hours in the oven at 103° C., and place in a vacuum for a few hours before weighing. Weigh in a glass-stoppered weighing bottle and record the weight of the tube and bottle consecutively. This weighing is done in advance of a slaughtering experiment, and several hundred tubes are prepared.

Place the finely ground and thoroughly mixed samples of meats in weighing bottles provided with short aluminum scoops (a heavy piece of stirring rod will do), and weigh by difference, using from 5 to 10 grams for a sample. Remove the cotton from one of the tared tubes, placing it on the side of a flat shallow porcelain dish, and carefully pour out the sand into the dish. Place the sample of the meat upon the sand and mix, using a spatula and a stirring rod. When the sand and sample are thoroughly mixed, transfer the mass to the tube, using the cotton to wipe all traces from the dish, the spatula, and the stirring rod. Loss of any particles of sand is prevented by working over black glazed paper. The last of the unused cotton is placed in the top of the tube as a plug.

Make the determinations in triplicate and place them in separate desiccators. (We use a good 6-inch vacuum desiccator. Larger desiccators were tried, but several of them broke, owing to the high pressure.) Wire-gauze baskets are used, which set on the porcelain desiccator plate. In this basket from eight to twelve tubes can be placed. The desiccator covers and stopcocks must be well ground and a lubricant

used which will hold and yet permit the easy removal of the cover. A mixture of 3 parts paraffin (hard) and 5 parts vaselin is satisfactory. These are melted together and then cooled slowly, with continual stirring. If the work is to be done during continued cold weather a little more vaselin may be used, or in summer a little more paraffin. The addition of rubber or Venice turpentine to the lubricant has been discontinued on account of the difficulty in removing the covers.

After the filled desiccators have been exhausted, rotate them carefully every three or four hours to mix thoroughly the acid and the water which has been absorbed into the upper portions. Care must be used not to spatter the acid upon the tubes. At the end of twenty-four to forty-eight hours, as is convenient, allow air to bubble slowly through concentrated sulphuric acid into the desiccator and transfer the tubes to a desiccator provided with fresh acid. Chemically pure sulphuric acid must be used, as the commercial acid discolors the samples.

Exhaust the freshly filled desiccators and hold for another twenty-four to forty-eight hours, as is convenient. During this interval mix the acid three or four times. Next weigh the tubes and place them in a vacuum again for twelve hours or longer and again weigh, to prove that the drying is complete. If any of the tubes do not show constant weight they are placed in vacuum again with fresh acid. The acid employed for the first drying is used for commercial acid; that with which the drying is completed is used as the first acid with fresh samples.

With blood the freshly drawn sample is rapidly poured into tared tubes filled with fat-free cotton, each tube being placed in a tared weighing bottle. The tube and stoppered bottle are weighed to get the weight of the sample, and the moisture is obtained as with meat samples.

We have demonstrated that this method is capable of practical application to agricultural analyses in general, and is especially to be recommended where a determination of the fat or ether-soluble constituents is to be made. The most marked differences have been noted in fat determinations upon samples of fresh bone (skeleton of beef). When heat has been used in drying the samples by the official method the extracted fats are frequently very dark colored. By using the vacuum method without heat the extracted fat is almost snow white. This method has been compared with the regular official method upon numerous other samples, as butter, milk, soil, feed stuffs, honey, soap, etc. A few results are given in the following tables illustrating several phases of the work.

*Moisture determinations on various animal substances by vacuum method without heat.*

Sample.	Results in triplicate.			Sample.	Results in triplicate.		
	(1)	(2)	(3)		(1)	(2)	(3)
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Blood.....	79.23	79.25	79.55	Round lean.....	72.67	73.22	72.61
Do.....	82.29	82.73	82.83	Kidney fat.....	5.52	5.51	5.42
Liver.....	68.77	68.74	68.96	Do.....	8.95	8.74	8.34
Do.....	68.82	68.82	68.41	Offal fat.....	12.51	12.29	12.43

*Moisture determinations on blood by vacuum method without heat.*

[Using absorbent cotton and showing effect of second drying in the vacuum.]

Weight of sample.	Weight of sample, tube, and weighing bottle.	First dry weight.	Second dry weight.	Loss in weight second time in vacuum.	Total loss in moisture.	Moisture.
<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>	<i>Per cent.</i>
6.4340	46.3238	41.1025	41.0984	0.0041	5.2254	81.216
3.7002	43.3378	40.3335	40.3324	.0011	3.0054	81.223
6.3934	44.0746	38.8884	38.8860	.0024	5.1886	81.156
5.3474	44.5125	40.1695	40.1685	.0010	4.3440	81.236

*Moisture determinations on a sample of liver, vacuum method without mixing with sand, showing gradual loss of moisture.*

Weight of sample.	Weight of container and fresh sample.	First dry weight.	Second dry weight.	Loss during second time in vacuum.	Third dry weight.	Loss during third time in vacuum.	Fourth dry weight.	Loss during fourth time in vacuum.	Fifth dry weight.	Loss during fifth time in vacuum.	Total loss in moisture.	Moisture.
Gms.	Grams.	Grams.	Grams.	Gm.	Grams.	Gm.	Grams.	Gm.	Gms.	Gm.	Gms.	Per ct.
6.9118	45.5373	40.7115	40.0977	0.0138	40.6875	0.0102	40.6845	0.0030	.....	.....	4.8528	70.210
7.1317	45.4085	40.4024	40.3888	.0136	40.3850	.0038	.....	.....	.....	.....	5.0235	70.439
8.0140	46.1368	40.5063	40.5435	.0568	40.5215	.0240	40.5121	.0094	40.5070	0.0051	5.6298	70.250

*Comparison of moisture determinations made by the vacuum method with those made by the official method.*

Sample.	By vacuum method.		By official method.	
	Per cent.	Per cent.	Per cent.	Per cent.
Wheat stubble, air dry.....	6.565	6.525	6.975	6.945
Soil, air dry.....	2.39	2.40	1.95	1.83
Corn chop, air dry <sup>a</sup> .....	13.54	13.57	13.36	12.88
Butter <sup>b</sup> .....	16.34	16.32	16.61	16.48
Cheese.....	32.96	32.97	33.44	33.25
Milk <sup>b</sup> .....	86.68	86.62	86.71	86.76

<sup>a</sup> The corn chop was placed in vacuum a fourth time before it ceased to lose in weight.

<sup>b</sup> The milk and butter were mixed with sand and were dry by the vacuum method at the end of twelve hours.

## THE UNIFICATION OF SACCHARIMETRIC OBSERVATIONS.

By C. A. BROWNE.

In an article upon the Control of Saccharimeters by Otto Schönrock,<sup>a</sup> it is shown that "the differences in rotation for sugar solutions disappear for different observers and different sources of white light only when the light is filtered through a 1.5 cm column of 6 per cent potassium bichromate solution in water." On the basis of these observations Schönrock recommends that the use of this light filter be adopted in defining the 100 point of the Ventzke scale. This recommendation, which has been followed by the Imperial Reichs Anstalt of Germany and the U. S. Bureau of Standards, seems, however, to be more or less disregarded by many chemists who work with saccharimeters.

The methods of the Association of Official Agricultural Chemists say nothing as to the use of potassium bichromate solution in saccharimeters, it being deemed perhaps a necessity too well known to require mention. We know, however, of chemists purchasing saccharimeters and using them for years blissfully ignorant of the presence of the empty cell in the end of their instruments or of the purpose for which the cell was intended to be used. Their mistake, which is due usually to inability to read the German directions which accompany the instrument, is perhaps pardonable. Less pardonable is the attitude of those chemists who, knowing of the cell and the purposes of its use, yet wilfully neglect it. One very common and most fallacious argument advanced against using the cell is that standardized quartz plates polarize correctly without it and that its use is therefore wholly unnecessary. Another reason given is that the bichromate renders the polarization of dark colored solutions so difficult that it is more convenient to eliminate it altogether.

<sup>a</sup> Zts. Ver. d. Zuckerind., 41: 521-58.

The directions of instrument makers as to the use of bichromate and the strength of solution to be employed are not at all explicit. One maker directs merely that the bichromate cell must always be filled with bichromate solution, which, however, can be more or less concentrated, according to the character of the liquid under examination.

The purpose of the bichromate solution in saccharimetric work is, of course, to correct the difference in rotation dispersion between cane sugar and quartz. The rays of light in the blue and violet which cause the greatest amount of rotation dispersion are absorbed by the bichromate. To gain more exact information as to the effect of eliminating the bichromate solution in the polarization of raw sugars I have recently compared the absorption spectra of bichromate solution with those of different clarified sugar solutions. Molasses sugars when clarified give a brownish yellow liquid, which absorbs practically all of the light in the blue and violet part of the spectrum. Solutions of such sugars act themselves as light filters and absorb the rays producing the greatest dispersion disturbances. They show upon polarization but little difference between filtered and unfiltered light. Clarified solutions of several low-grade beet sugars were found to absorb all of the violet but only a part of the blue. Slight rotation dispersion was obtained without the bichromate cell. Ninety-six degree centrifugal sugars give usually straw-colored solutions, which absorb most of the violet, but practically nothing of the blue. Rotation dispersion with these sugars is usually well marked without bichromate. Java and other high-grade sugars give upon clarification nearly colorless solutions which show very pronounced rotation dispersion without the bichromate. With such sugars the difference in reading with and without bichromate was in some cases nearly 0.2 per cent for the same observer.

The error due to rotation dispersion was found by Schönrock to be variable with different observers, a circumstance due perhaps to some physiological difference in the pigment of the eye. Comparisons which I have made on five sugars polarizing over  $96^\circ$ , using no bichromate and 1 and 3 per cent solutions of bichromate in a 3 cm cell, showed that the discrepancies in the readings of the same solution between four observers were augmented six and one-half times, when no bichromate was used, as compared with the 3 per cent bichromate, and two and one-half times when 1 per cent bichromate was used, as compared with the 3 per cent. Using a 3 per cent solution of bichromate in a 3 cm cell the average difference between the readings of the lowest of the four observers and the other three was only  $0.03^\circ V.$ , using a 1 per cent solution the average difference was  $0.08^\circ V.$ , and using no bichromate  $0.22^\circ V.$  The 3 per cent bichromate in a 3 cm cell gives the same effect as the 6 per cent bichromate in a 1.5 cm cell advocated by Schönrock. The use of bichromate of the above concentrations according to the length of cell should therefore be prescribed and rigidly adhered to in the polarization of sugars.

These concentrations apply, however, only to cane sugar. With substances of greater rotation dispersion such as commercial glucose, dextrin, malt products, etc., it will be found necessary to increase the strength of the bichromate considerably, as may be seen from the following:

*Polarizations of starch conversion products with and without bichromate ( $^\circ V.$ ).*

Starch conversion products.	No bichromate.	Strength of bichromate, 3 cm cell.		
		0.5 per cent.	3 per cent.	6 per cent.
Dextrin.....	253.65	253.50	253.40	253.00
Malt sirup.....	195.80	195.50	195.40	195.15
Glucose sirup.....	179.90	179.70	179.70	179.55
Do.....	172.10	171.85	171.75	171.55

With starch conversion products it is possible to secure concordant readings between different observers only when 6 per cent bichromate is used in a 3 cm cell. With substances of higher dispersion than dextrin it would seem advisable to use only sodium light for polarization. With all carbohydrate materials it would seem that the dispersion disturbances of white light may be eliminated by means of bichromate solution. The results show, however, that the directions for operating saccharimeters should specify the exact strength of bichromate solution to be used.

A second and very discordant element in the unification of saccharimetric observations is in the use of clarifying agents. The several errors resulting from the use of lead salts in clarifying sugar solutions have long been recognized. There is, first, the volume of precipitate error; second, the precipitation of levulose error; third, the formation of soluble lead levulosate of lower specific rotation than levulose; and, fourth, when dry defecation is used, the error of dilution or change in volume.

In studying these various questions my attention was directed first of all to the great difference in composition of the commercial preparations of lead subacetate and also of the solutions of this salt as ordinarily prepared for laboratory use. Preparations of the anhydrous subacetate of lead sold by reliable chemical firms, and all guaranteed as to purity according to the food and drugs act, were found to vary in their content of basic lead oxid from 3.34 to 32.32 per cent. Solutions of lead subacetate prepared by digesting litharge with the normal acetate of lead, according to the method of the association or other directions, will also vary greatly in composition, according to the time and temperature of digestion. Solutions of the same specific gravity thus prepared were found to vary in the ratio of combined to basic PbO of from 5:2 to 1:1. These variations in composition are not surprising when it is remembered that three well-defined subacetates have been prepared by the digestion of litharge with normal lead acetate. These are  $3\text{PbAc}2\text{PbO}$ , the subacetate ordinarily prescribed for clarification;  $\text{PbAcPbO}$ , the monobasic acetate; and  $\text{PbAc}2\text{PbO}$ , the dibasic acetate.

The official directions for preparing basic lead acetate are explicit as to the specific gravity of lead solutions to be used, but are silent as to the point of greatest importance, the content of basic lead. The differences which may result in saccharimetric work from the use of lead solutions of varying basicity may be seen from the following polarizations made upon a sirup and a sugar using three different solutions of lead subacetate and one solution of the normal acetate all of 1.24 specific gravity.

*Comparison of polarizations using different solutions of lead subacetate.*

Material	Quantity of reagent.	Normal lead acetate.	Lead subacetate.		
			$5\text{PbAc}2\text{PbO}$	$3\text{PbAc}2\text{PbO}$	$\text{PbAcPbO}$
	cc.	° V.	° V.	° V.	° V.
Sirup.....	8	45.00	45.50	45.65	46.00
Sugar.....	10	81.85	82.30	82.40	82.50

The solutions of greatest basicity have the greatest clarifying power and give the highest polarizations owing to the greater precipitation and lowering of polarization of the levulose and consequent increase in dextro-rotation. The 3:2 subacetate is the one usually prescribed, and since this compound can be obtained of satisfactory purity from one chemical house at least it might be well for chemists desiring uniformity to prepare these solutions directly from this salt. The important point, however, is that in whatever way prepared the solutions of basic lead used in saccharimetry should have not only a constant specific gravity, but a uniform content of basic lead.

The errors due to the volume of lead precipitate, a most serious one in the polarization of low-grade saccharine products, have been very largely eliminated by the ingen-

ious method of dry defecation proposed some years ago by W. D. Horne. The questions of change in volume and precipitation of levulose, when the dry subacetate is used in large amounts, as is always necessary with low-grade products, have given rise, however, to some uncertainties, and the method has not met with universal approval. To determine exactly the amount of error due to change in volume and precipitation of levulose, mixtures of sucrose and invert sugar, with mineral and organic salts precipitable by lead, were prepared and the effects produced upon the polarization of these solutions by different quantities of the dry acetate and dry subacetate of lead noted. It was found that in quantities up to 0.5 gram but very little change could be detected in the polarization of the original solution when either the dry acetate or dry subacetate of lead was used. Using more than 0.5 gram of substance the dry acetate invariably reduced the polarization owing to the increase in volume produced by the dissolved salt; the effect of increased quantities of the dry subacetate, however, was variable. Where but little invert sugar was present there was the same decrease in polarization owing to increase in volume. Where considerable invert sugar was present, however, this dilution error was counterbalanced, and often more than counterbalanced, by the precipitation and lowering of the specific rotation of the levulose and there was either no change in the reading of the original solution or an increase. Used in very large excess beyond the precipitation of the levulose the dry subacetate produced a continuous lowering of the polarization through dilution.

The same facts were noted in connection with the polarization of commercial sugars, as may be seen from the following polarizations made in New York by M. H. Wiley:

*Effect produced by different quantities of acetate and subacetate.*

Sample.	Subacetate solution.		Dry lead acetate.				Dry subacetate.			
	cc.	° V.	Gram.	° V.	Gram.	° V.	Gram.	° V.	Gram.	° V.
1. Java sugar.....	1	98.10	0.3	97.95	2.0	97.65	0.5	97.90	2.0	97.75
2. Philippine mats.....	3	88.95	1.5	88.45	3.0	88.15	1.5	88.65	3.0	88.65
3. Cuba molasses.....	1	90.75	0.5	90.60	2.0	90.40	0.5	90.65	2.0	90.55
4. Do.....	4	86.15	2.0	85.55	4.0	85.30	2.0	85.70	4.0	85.70

The dry normal acetate by the addition of excess produced dilution in every instance as is seen by the diminished polarization. This same dilution is noticed by the dry subacetate, but to a much less extent on samples 1 and 3; on samples 2 and 4 doubling the quantity of dry lead subacetate caused no change in the polarization through the compensating effect of the levulose precipitation. It is needless to add that the double quantity of lead used was beyond that necessary to secure clarification, so that an idea may thus be formed of the probable errors due to excess.

In some interesting clarification experiments by J. A. Hall in the New York Sugar Trade Laboratory the effect of adding varying amounts of dry lead subacetate was studied in another way. Starting with a minimum quantity of the salt, this amount was increased and the effect upon the polarization and the amount of lead dissolved in the clarified filtrate noted. By calculating the dissolved lead to the subacetate it is possible to estimate the dilution, allowing 0.22 cc increase of volume to 1 gram of subacetate as determined by Horne. Only one experiment upon a No. 2 Philippine mat sugar is cited:

*A second comparison of effects of varying quantities of clarifying agents on dilution and polarization.*

Clarifying agent.	In 100 cc filtrate.		Estimated dilution.	Polarization.
	PbO.	Pb subacetate.		
Subacetate (cc) <sup>a</sup> .....	3.0	Grams. 0.2678	cc.	° V. 86.70
Dry subacetate (grams).....	0.5	Trace.	Trace.	Too dark to read.
Do.....	1.0	1530	(0.20)	86.50
Do.....	2.0	7203	(0.94)	86.60
Do.....	4.0	2.1078	(2.73)	86.50

<sup>a</sup> Sp. gr. 1.250.

It will be noted that with an estimated dilution of 0.2 cc instead of a decrease in polarization as would be expected there is an increase. With an estimated dilution of 0.6 cc the reading is the same as that first obtained, so that the combined effect of the dry lead upon the precipitation of levulose and upon the lowering of the rotation of the levulose in solution is seen to be most pronounced. It will be noted that when an excess of dry lead is added not all of this passes into solution. Adding 1 gram excess caused an increase in the filtrate of only 0.74 gram, and 2 grams an increase of only 1.8 grams. After the solution is sufficiently clarified for reading addition of more lead will continue to form a precipitate, so that the rule of adding lead until no more precipitate forms is not always a safe one to follow. An interesting fact in this connection is that the addition of much lead subacetate beyond the point of maximum clarification for low-grade cane products will produce a darkening of the solution. This is due to the well-known color reaction between reducing sugars and alkalis.

If the minimum amount of dry lead subacetate necessary to secure satisfactory clarification be carefully determined for each grade of commercial product and excess beyond this be avoided there is no question but what this method of clarification gives polariscopic readings closer to the true polarization than any other method thus far proposed. The use of dry lead subacetate and subacetate solution as defecating agents in the determination of reducing sugars should of course be avoided.

A third and one of the greatest causes of the lack of agreement in saccharimetric observations between different chemists is variation in temperature. As regards the effect of temperature upon the polarization of pure sucrose nearly all chemists are in very close agreement. For quartz-wedge saccharimeters the researches of Andrews, Wiley, Schönrock, Watts and Tempany, and other chemists show that for each degree Centigrade increase in temperature there is a falling off in the polarization of pure sucrose of about 0.031° Ventzke. The question now arises, with this variation in the specific rotation of sucrose with temperature, what correction, if any, should be applied to the polarization of commercial products.

In my report as associate referee on sugar, made to the association<sup>a</sup> in 1905, it was shown that the application of temperature corrections to low grade cane sugars was not advisable, for the reason that the polarization of a sugar is an expression not merely of the sucrose alone but of all the optical constituents present and since some of these optical constituents, more especially the levulose, are affected by temperature in a manner contrary to sucrose, it is not permissible to make a temperature correction for one constituent without at the same time correcting for the others.

This view of the question has been recently contested by Dr. Francis Watts, government chemist, and Mr. H. A. Tempany, assistant government chemist, for the Leeward Islands of the British West Indies, in a recent number of the West Indian Bulletin.<sup>b</sup>

<sup>a</sup> U. S. Dept. of Agr., Bureau of Chemistry, Bul. 99, p. 20.

<sup>b</sup> 1908, 9: 127.

They advocate for the purpose of securing greater uniformity and exactness among analysts the application to all polarizations of a correction formula " $N + 0.00031 tN$ ", where  $N$  is the observed reading on the Ventzke scale and  $t$  is the difference between the temperature of observation and that at which the polarimeter was standardized." In answer to my criticisms of such a correction when applied to raw cane sugars Messrs. Watts and Tempany reply as follows:

While not disputing the accuracy of the statement concerning the effect of temperature on levulose, we would point out that the process of determining the polariscopic test of a sugar is purely arbitrary and conventional. We take it that the polariscopic test of any sample of sugar is the rotation produced by it when tested in such a way that a sample of chemically pure sucrose tested under precisely similar conditions would give a reading of  $100^\circ$ . The 100 point of the Ventzke, or any other sugar scale, is based on the rotation of a standard weight of sucrose, dissolved in a standard volume of water, at a standard temperature. If at any other temperature this weight of pure sucrose will not give a rotation of  $100^\circ$  on the scale, the scale has been altered; consequently, allowance must be made for this alteration in the scale when polarizing commercial sugars under these conditions.

The above criticism of my previous article is, however, not a valid one. We could say with equal justice: Consequently, allowance must be made for this alteration in the scale when polarizing molasses or honey or condensed milk or glucose or any other substance which is polarized upon a saccharimeter. The only scientific conclusion which could be drawn is—allowance must therefore be made for this alteration in the scale when polarizing pure sucrose; to include commercial sugars and other substances is too sweeping and unwarranted a generalization. It is true that the 100 point of the sugar scale of a saccharimeter is based upon the rotation of a standard weight of c. p. sucrose under certain standard conditions; this sucrose, however, is a means of standardization and nothing more. A definite weight of milk sugar can be made to read 100 upon any saccharimeter and this weight is used for the estimation of milk sugar in milk products. To apply a correction formula for sucrose in such cases would of course be an absurdity.

Quartz may also be used for standardization, and is so used, the 100 point of the French sugar scale being based upon the rotation of a plate of quartz 1 mm thick. It might be said, following the same line of argument as that of Messrs. Watts and Tempany, that because a standard plate of quartz always polarizes  $100^\circ$  irrespective of temperature upon a quartz-wedge saccharimeter, the scale has not been altered and consequently no allowance at all should be taken of temperature in the work of polarization, a conclusion of course perfectly true as regards quartz but not of other substances. Similarly the conclusions worked out for chemically pure sucrose for a given type of saccharimeter are true for chemically pure sucrose but for nothing else, neither for mixtures of sucrose with other substances nor for products which contain no sucrose.

The International Commission for Uniform Method of Sugar Analysis in 1900 decided that it was permissible, as in tropical countries, to adjust saccharimeters to a higher standard temperature than  $20^\circ$  C. This adjustment may be made by changing the quartz wedges of the instrument, by increasing the normal weight of sugar, by increasing the length of the observation tube, or in other ways. When only local comparisons are involved it is advisable and advantageous to make such an adjustment; there is a serious objection, however, against having several separate standards for universal work, since comparisons are no longer possible upon a large class of low-grade saccharine products. Two saccharimeters, for example, one standardized for the rotation of sucrose at  $20^\circ$  and one standardized for the rotation of sucrose at  $30^\circ$ , will give, of course, identical results for pure sucrose, but not for a raw cane sugar, nor for a cane molasses, nor for a large class of other products. Having adjusted our saccharimeter to any desired standard temperature, this standard temperature must be rigidly adhered to if identical observations are to be always obtained between different chemists.



The true polarization then of a raw sugar, as of other saccharine products, is a conventional arbitrary figure representing the sum of the polarizations of the various optical constituents under certain fixed conditions of temperature, weight of substance, volume of solution, length of tube, and quality of light. If the temperature of polarization of a given sugar is different from the standard the correction, if correctly applied, must restore the reading obtained upon this same sugar under standard conditions. Now, the correction advocated by Watts and Tempany and that used by the United States Treasury Department in the Division of Customs will do this for pure sucrose, but it will not do it for a very large class of raw cane sugars for the reasons already given.

Since the publication of my previous paper upon this subject I have had occasion to study the effect of temperature upon the polarization of many sugars and other cane products and have been more thoroughly convinced than ever of the futility of applying such a correction for the purpose of securing greater concordance in the saccharimetric observations of different chemists.

The general results of this work I have condensed into tabular form, showing the ranges of polarization and of reducing sugars for raw cane sugars, and for several types of masseccutes and molasses with the corrections necessary to obtain the polarization at standard temperature. The theoretical sucrose corrections according to the formula of Watts and Tempany are appended for purpose of comparison. The values of the table have been made up from averages, some variation was obtained for individual classes of raw sugars, as, for example, those of Louisiana which are very high in reducing sugars and give a correspondingly lower correction. It is believed, however, that the table, on the whole, is a fair average.

Table for correcting polarizations of raw cane sugars, etc., to standard temperature.

[Correction for each °C. above standard temperature.]

Polarization.	Reducing sugars.	Actual correction.	Correction by formula 0.00031 P.
SUGAR.			
° V.	Per cent.	° V.	° V.
100-96	0.00-1.00	+0.028	+0.030
96-94	1.00-1.60	+0.024	+0.029
94-92	1.60-2.20	+0.021	+0.029
92-90	2.20-3.00	+0.017	+0.028
90-88	3.00-3.80	+0.014	+0.028
88-86	3.80-4.60	+0.009	+0.027
86-84	4.60-5.40	+0.005	+0.026
84-82	5.40-6.20	+0.002	+0.026
82-80	6.20-7.00	-0.003	+0.025
80-78	7.00-7.80	-0.007	+0.025
78-76	7.80-8.60	-0.011	+0.024
76-74	8.60-9.00	-0.014	+0.023
MASSECCUTE.			
68-72	3.00-10.00	-0.016	+0.022
58-62	12.00-14.00	-0.036	+0.019
44-48	16.00-18.00	-0.057	+0.014
MOLASSES.			
34-30	18.00-20.00	-0.070	+0.010
22-26	24.00-26.00	-0.098	+0.007
16-20	28.00-30.00	-0.116	+0.006

It will be noted that for very high-grade sugars which polarize over 96 an addition of about 0.03° V. for each ° C. increase in temperature will practically restore the reading obtained under standard conditions. The percentage of impurities is too small to affect appreciably the temperature correction for sucrose. As the polarization falls below 96 and the percentage of reducing sugars increases, the effect of the tempera-

ture upon the rotation of the levulose begins to lower the theoretical sucrose correction, until at a point usually about 80 to 86 the two influences—that of the temperature upon the levulose and other impurities and that of the temperature upon the sucrose and quartz wedges of the instrument—counterbalance one another. Two chemists polarizing such a sugar, one working at 30° C. and one working at 20° C., other conditions being equal, will obtain concordant and correct readings; the application of the theoretical sucrose correction would place the observation of the chemist working at 30° C., 0.25° V. too high.

Below 80 the effect of increase in temperature is usually to elevate rather than diminish the reading, this influence becoming more and more pronounced in the masseccutes and molasses; the levulose correction more than counterbalances the theoretical one due to sucrose. Every chemist knows how pronounced this influence is on the polarization of sirups and molasses, how the simple handling of the observation tubes will increase the readings. It is the same with low-grade sugars which consist simply of sucrose crystals contaminated with varying amounts of molasses. When such sugars are polarized above 20° C. a correction would have to be subtracted to secure the reading that would be obtained under standard conditions. To add a correction, as required by a sucrose correction formula, would manifestly only further increase the error of observation.

The solution of the temperature question then resolves itself simply into this: If we are to make temperature corrections in the polarizations of commercial products, we must correct for variations in the specific rotation of all the ingredients therein present. If it is impossible to do this, no temperature corrections at all should be applied; instead of this we should strive to make our polarizations as nearly as possible under standard conditions. Custom-house laboratories, arbitration laboratories, and all other laboratories, upon the results of which great interests are involved, should be equipped with cooling and warming apparatus for maintaining a constant uniform standard temperature. The great testing laboratories of Germany are so provided and similar institutions in this country should do as much. For chemists who are unable to provide themselves with this equipment much can be done by moving the laboratory to cooler quarters, as from a hot upper room to a cool basement. By such a change the New York Sugar Trade Laboratory has lowered the temperature of testing from 25° C. to 21.5° C. in hot weather.

The services rendered to science by the researches of the many chemists who have investigated the influence of temperature upon the specific rotation of sucrose are great; the results of their labors are lasting and will stand the test of time. The application, however, of what they have established for pure sucrose to the polarization of all grades of saccharine products is a misapplication. It is a great mistake. It will increase rather than diminish the errors between many of the saccharimetric observations of different analysts and is bound to work great injustice when applied commercially.

A paper on the influence of glycerin, acetanilid, and certain other drugs in the estimation of alcohol by L. E. Warren and H. C. Fuller of the Division of Drugs, Bureau of Chemistry, was presented by Mr. Warren. This work, bearing especially upon the drug investigations, has been printed elsewhere for greater accessibility.<sup>a</sup>

The associate referee presented a lengthy paper by S. H. Baer on the colorimetric method for the determination of citral, dealing largely with the chemistry of that substance. The portions on criticisms of the method are reported in abstract.

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<sup>a</sup> Amer. J. Pharm., 1909, 81: 66.

## CITRAL AND ITS ANALYSIS IN TERPENELESS EXTRACT OF LEMON.

By SAMUEL H. BAER.

The analyses were made by three chemists, including the writer, and as all three judged the colors, it would seem that the analyses are as accurate as the colorimetric method permits. Acknowledgment is due S. E. Shaffner for assistance rendered.

### *Determination of citral in lemon extract by the colorimetric method.*

Sample No.	Description.	Citral.	
		Estimated amount present.	Amount found.
		<i>Per cent.</i>	<i>Per cent.</i>
10	Terpeneless oil of lemon solution (dissolved in cologne spirits, 190 proof, or 95 per cent, and colored with lemon peel).....	0.42	0.19
11	Terpeneless oil of lemon solution (dissolved in cologne spirits, 190 proof, or 95 per cent, and colored with turmeric).....	.42	.18
12	Terpeneless oil of lemon solution (dissolved in 38 per cent cologne spirits and city water and filtered through magnesia).....	.42	.10
13	Citral solution (dissolved in cologne spirits of 190 proof, or 95 per cent).....	.42	.40
14	17 pounds oil of lemon, 19 gallons cologne spirits, 23 gallons water (colored with lemon peel and filtered through magnesia).....		.10
15	Alcohol (not cologne spirits, 188 proof, generally used by manufacturers).....		.19
16	Cologne spirits, 190 proof.....		.07
17	50 per cent cologne spirits with city water.....		.07
18	50 per cent cologne spirits filtered through magnesia.....		.08

From these analyses it is seen that when the colorimetric method is applied to the extracts of commerce, the correct result is not obtained. On sample No. 13, a citral solution, the analysis was reasonably close; samples No. 10 and 11 are terpeneless oils of lemon and the low results on citral may be due to the fact that the sample purchased was not pure terpeneless oil of lemon, but a product containing only 50 per cent of the citral that should be there.

Most of the extract manufacturers use 188 proof alcohol, that is, 94 per cent alcohol, which always contains a certain amount of aldehydes, and the sample used in this test, treating the alcohol the same as the lemon extract, showed 0.19 per cent of citral, when there was no citral there at all. If only cologne spirits are used, the results obtained are not so far wrong as if 94 per cent alcohol is used.

Since, therefore, the presence of the impurities in alcohol throw the results off to such an extent, giving too high a per cent of citral, would it not be possible that the impurities in the alcohol at certain times and also in the water, and the very change of one or two ingredients in the lemon oil, might make the result inaccurate, reversing the analysis and showing a smaller per cent of citral than is really present?

The colorimetric method is applicable if the manufacturer used chemically pure citral, distilled water, and aldehyde-free alcohol in the manufacture of his extracts, but such ideal conditions never exist. Further, any manufacturer could discreetly add another aldehyde, even acetaldehyde, to the extent of 0.2 per cent, which would give all the reactions of citral in the extract of lemon by the colorimetric method.

The method is not without use, but if the presence of citral could be determined and estimated quantitatively by a sodium sulphite or carbazone method, then the colorimetric method might be used as a check. Before adopting the colorimetric method as official a committee should be appointed from the association members to test it carefully, under the conditions that the manufacturer must meet since he can not use aldehyde-free alcohol, nor is he always in a position to use distilled water.

Further, suppose the method is accurate, how would the analyses show that the citral used was obtained from lemon oil or the commercial citral obtained from lemon grass oil?

## AN OUTLINE TO ASSIST IN THE IDENTIFICATION OF CERTAIN WATER-SOLUBLE COAL-TAR COLORS.

By C. B. COCHRAN<sup>2</sup>.

The reactions given by the coal-tar colors listed in the following outline were all obtained with solutions as dilute as they could be made and still give reactions sufficiently clear and definite to furnish a basis for positive conclusions. Because of the degree of dilution the results here tabulated will, in some cases, appear contradictory to those given by Schultz and Julius. For example, these authors may report a color precipitated by a certain reagent when the precipitation is only partial and therefore does not appear in dilute solutions such as have been used in the preparation of these tables.

The sodium bisulphite reagent is prepared by saturating a 5 per cent solution of sodium hydroxid with sulphur dioxid. The absorption tests with aluminum hydroxid were made by adding between 2 and 3 cc of well-washed aluminum hydroxid (from which the excess of water has been drained through the filter) to 10 cc of the color solution.

The tests with the fuller's earth were made by adding 2 cc of the earth to 10 cc of the color solution. In these absorption tests the aluminum hydroxid and fuller's earth are shaken with the color solution. If, after setting, the supernatant liquid is colorless or very nearly so, the result is recorded as color absorbed. In the majority of cases the results obtained with aluminum hydroxid and fuller's earth are definite and sharp. There are many colors belonging to Class I (Rota's classification) which are much more readily absorbed from their water solutions by aluminum hydroxid than by fuller's earth, while the reverse is true of many colors belonging to Classes II, III, and IV.

In the dyeing tests sodium carbonate was used for making alkaline and hydrochloric acid for acidifying. The alkali solution was very weak and the acid bath about one-half the official strength (1 cc strong hydrochloric acid to 50 cc).

The numbers following the names of the colors refer to the 1904 edition of Green's tables.

### COAL-TAR COLORS OF CLASS I.

Solution reduced and in most cases decolorized by stannous chlorid. Original color not restored by hydrogen dioxid.

#### DIVISION I.—COLOR ABSORBED BY ALUMINUM HYDROXID.

##### *Dye wool red.*

SECTION I.—Color precipitated by sodium bisulphite reagent.

Congo red (A) (240) dyes wool and unmordanted cotton red from neutral or faintly alkaline bath, but not from acid bath. Oxalic acid or acetic acid gives a blue precipitate and colorless filtrate.

SECTION II.—Color not precipitated nor solution changed by sodium bisulphite reagent.

Fast red A (102), hydrochloric acid gives a brown precipitate and colorless filtrate. Dyes wool and unmordanted cotton red from acid, alkaline, or neutral bath. Color precipitated by barium chlorid solution.

Azo rubin S (103), color only partially precipitated by hydrochloric acid. Dyes wool red from acid bath but not from alkaline bath. Does not readily dye unmordanted cotton in either bath. Color not precipitated by barium chlorid.

*Dyes wool yellow.*

Chrysamin R (269), hydrochloric acid gives a brown precipitate, sodium hydroxid a red solution. Barium chlorid and sodium bisulphite reagent each gives a yellow precipitate and colorless filtrate. Dyes wool pale yellow from a neutral bath and unmordanted cotton orange yellow from a neutral or alkaline bath.

*Dye wool and unmordanted cotton brown from acid bath.*

Bismarck brown (197), decolorized by stannous chlorid and on adding hydrogen dioxid a color somewhat redder than the original color appears. Color precipitated by tannin reagent. Color absorbed from alkaline solution by ether, and on adding dilute acetic acid to the ether solution, the color is taken up by the acid.

Resorcin brown (137), decolorized by stannous chlorid. No color returns on adding hydrogen dioxid. Not precipitated by tannin reagent. Color absorbed by fuller's earth.

**DIVISION II.—COLOR NOT ABSORBED OR ONLY PARTIALLY ABSORBED BY ALUMINUM HYDROXID.**

*Dye wool red in acid bath.*

**SECTION I.**—Sodium hydroxid causes a distinct change in color of water solution.

(1) Sodium hydroxid turns water solution violet.

Ponceau 6 R. B. (169), dyed wool is bluish red, turned blue by hydrochloric acid or sulphuric acid, color in wool dissolves in either acid, giving a blue solution. Hydrochloric acid turns water solution violet, more turns it blue.

(2) Sodium hydroxid turns water solution brown.

Brilliant crocein (146), hydrochloric acid produces little change in color of water solution.

Crystal ponceau (A) (64), dyed wool turned violet by hydrochloric acid and blue by sulphuric acid.

Crocein scarlet 3 B X (104), dyed wool turned red violet by hydrochloric acid or by sulphuric acid.

New coccein (A) (106), color of dyed wool not changed by hydrochloric acid.

(3) Sodium hydroxid turns water solution yellow.

Palatin scarlet (53), dyed wool is scarlet. Color not much changed by hydrochloric acid, but sulphuric acid turns it violet and gives a violet solution. On dilution wool has nearly original color.

**SECTION II.**—Sodium hydroxid does not cause a distinct change in the color of the water solution.

*Group I.*—Sulphuric acid turns dyed wool blue or violet and gives a blue or violet solution. Dyed wool is bluish red.

Bordeaux B (A) (65), hydrochloric acid turns dyed wool violet.

Bordeaux S (A) (107), scarlet B. E. E. (P) closely related to Bordeaux S.

*Group II.*—Sulphuric acid has little or no effect on color of dyed wool. Dyed wool is scarlet.

Ponceau G (A) (55), barium chlorid gives an orange red precipitate, wool dyed orange red.

Ponceau 3 R (A) (56), barium chlorid gives a red precipitate. Dyes wool more red than (55).

*Dye wool yellow or orange.*

SECTION I.—Hydrochloric acid added to strong acidification precipitates the color or decolorizes the solution (the nitro colors).

*Group I.*—Color extracted by ether from solution acidified with hydrochloric acid. Victoria yellow (2).

Martius yellow (3), water solution plus potassium cyanid gives a brown color on warming.

*Group II.*—Color not extracted by ether from solution acidified with hydrochloric acid.

Naphthol yellow S (4).

SECTION II.—Hydrochloric acid causes a decided change in the color of the water solution (many of the tropæolins).

*Group I.*—Hydrochloric acid turns dyed wool violet.

Dyed wool is yellow.

Brilliant yellow S (Sch.) (89), dyed wool is yellow turned violet by hydrochloric acid.

Metanil yellow (Sch.) (95), dyed wool is orange yellow turned violet by hydrochloric acid.

*Group II.*—Hydrochloric acid turns dyed wool brown.

Chrysoidin R (18). This color is absorbed by fuller's earth and partially absorbed by aluminum hydroxid.

*Group III.*—Hydrochloric acid turns dyed wool red.

Fast yellow (8).

SECTION III.—Color of water solution not decidedly changed by hydrochloric acid. (If a precipitate appears only a part of the color is precipitated.)

Dyed wool is yellow.

Naphthol yellow S (4), dyed wool is decolorized by hydrochloric acid.

Tartrazin (94), color of dyed wool not changed by hydrochloric acid.

Dyed wool is yellow orange to orange.

Tropæolin O (84).

Tropæolin OOO (85).

Orange G (14).

Dyed wool is red orange.

Mandarin G (86), dyed wool is turned red violet by hydrochloric acid or sulphuric acid.

Ponceau 4 G. B., color of dyed wool not changed by hydrochloric acid nor by sulphuric acid.

#### COLORS OF CLASS II.

Solution decolorized by stannous chlorid, original color returns on addition of hydrogen dioxid. (Bismarck brown, which might be referred to this class, is included under Class I.)

(1) Dyes wool and cotton bluish red (most readily from an alkaline bath).

Safranin (584), much hydrochloric acid turns water solution blue violet. Color absorbed by fuller's earth, precipitated by tannin reagent. Sulphuric acid turns dyed wool green, solution green, hydrochloric acid blue.

(2) Dyes wool blue from alkaline or neutral bath, cotton a paler blue from neutral bath.

Methylene blue (650), color absorbed by fuller's earth precipitated by tannin; hydrochloric acid turns dyed wool robin's-egg blue, sulphuric acid green.

#### COLORS OF CLASS III.

Stannous chlorid produces no further effect on the color than hydrochloric acid. Sodium hydroxid produces a precipitate or decolorizes the solution. All the colors given in this class except auramin (425) are decolorized by sodium bisulphite reagent.

The color reappears on heating and disappears on cooling. With the exception of acid magenta (A) (462) they are all absorbed by fuller's earth.

*Dye wool red.*

(1) Dye wool from acid bath only, do not dye unmordanted cotton in either bath.

Acid magenta (462), color absorbed by aluminum hydroxid. Dyed wool is decolorized by hydrochloric acid, sulphuric acid, sodium hydroxid, or ammonium hydroxid. Tannin reagent gives no precipitate.

(2) Dyes wool and also unmordanted cotton most readily from a neutral bath.

Fuchsin (448), color not absorbed by aluminum hydroxid. Dyed wool turned red brown by hydrochloric acid or sulphuric acid. Tannin reagent gives a precipitate.

(3) Dyes wool yellow from neutral or alkaline bath. Does not dye unmordanted cotton. Aurantin (425).

*Dye wool green.*

Dye from acid bath: Guinea green B (A) (433) and acid green (434) do not dye cotton.

Dye from neutral or alkaline bath: Ethyl green (428) dyes unmordanted cotton more readily than malachite green.

Malachite green (427), dyed wool is blue green, turned at first grass green by hydrochloric acid or sulphuric acid, then yellow; on dilution, blue.

*Dye wool violet from neutral or alkaline bath.*

Methyl violet (451), sodium hydroxid gives a brown precipitate and brown solution.

Ethyl violet (453), sodium hydroxid gives a white precipitate, colorless on warming. Either dyes unmordanted cotton from alkaline bath.

*Dyes wool blue from acid bath.*

China blue (480), color absorbed by aluminum hydroxid. Solution decolorized by sodium bisulphite reagent. Color does not readily return on heating, but does return on adding a drop of hydrochloric acid. Dyed wool decolorized by ammonium hydroxid, turned reddish brown by sulphuric acid.

COLORS OF CLASS IV.

Colors not reduced by stannous chlorid. Solution not decolorized and color not completely precipitated by sodium hydroxid.

*Dye wool red from neutral bath.*

Dyed wool is red orange to orange red:

Eosin (512), color not absorbed by fuller's earth nor by aluminum hydroxid. Water solution yellow to orange with green fluorescence. Hydrochloric acid or sodium bisulphite reagent gives an orange precipitate.

Dye wool bluish red from neutral bath:

(a) Color completely absorbed by fuller's earth. Sodium bisulphite reagent gives no precipitate, but causes only a loss of fluorescence.

Rhodamin G (502), water solution red violet with red fluorescence.

Rhodamin B (504), water solution bluish red with orange brown fluorescence.

(b) Color only partially absorbed by fuller's earth. Sodium bisulphite reagent precipitates the color.

Erythrosin (516), water solution cherry red. (Green's tables give no fluorescence. A sample marked "Grübler" gave green fluorescence.) Hydrochloric acid gives an orange brown precipitate. Sodium bisulphite reagent gives an orange-red precipitate.

Rose bengal (520), water solution cherry red. No fluorescence. Hydrochloric acid gives a brown-red precipitate. Sodium bisulphite reagent a pink precipitate.

Phloxin (521), water solution bluish red with green fluorescence. Hydrochloric acid gives an orange precipitate. Sodium bisulphite a pink precipitate.

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Chemical methods: A. H. Bryan, Washington, D. C.

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*Inorganic plant constituents:* O. M. Shedd, Lexington, Ky.

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Committee B: E. M. Chace (3), F. W. Woll (2), chairman, Agricultural Experiment Station, Madison, Wis.; F. P. Veitch (1).

Committee C: C. D. Howard (3), A. L. Winton (2), chairman, U. S. Food Inspection Laboratory, Chicago, Ill.; L. M. Tolman (1).

## CONSTITUTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

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(1) This association shall be known as the Association of Official Agricultural Chemists of North America. The objects of the association shall be (1) to secure uniformity and accuracy in the methods, results, and modes of statement of analysis of fertilizers, soils, cattle foods, dairy products, and other materials connected with agricultural industry; (2) to afford opportunity for the discussion of matters of interest to agricultural chemists.

(2) Analytical chemists connected with the United States Department of Agriculture, or with any State, Provincial, or National agricultural experiment station or agricultural college, or with any State, Provincial, or National institution or body in North America charged with official control of the materials named in section 1, shall alone be eligible to membership; and one such representative for each of these institutions or boards, when properly accredited, shall be entitled to enter motions or vote in the association. Only such chemists as are connected with institutions exercising official fertilizer control shall vote on questions involving methods of analyzing fertilizers. All persons eligible to membership shall become members ex officio and shall be allowed the privileges of membership at any meeting of the association after presenting proper credentials. All members of the association who lose their right to such membership by retiring from positions indicated as requisite for membership shall be entitled to become honorary members and to have all privileges of membership save the right to hold office and vote. All analytical chemists and others interested in the objects of the association may attend its meetings and take part in its discussions, but shall not be entitled to enter motions or vote.

(3) The officers of the association shall consist of a president, a vice-president, and a secretary, who shall also act as treasurer; and these officers, together with two other members to be elected by the association, shall constitute the executive committee. When any officer ceases to be a member by reason of withdrawing from a department or board whose members are eligible to membership, his office shall be considered vacant, and a successor may be appointed by the executive committee, to continue in office till the annual meeting next following.

(4) There shall be appointed by the executive committee, at the regular annual meeting, from among the members of the association, a referee and such associate referees for each of the subjects to be considered by the association as that committee may deem appropriate.

It shall be the duty of these referees to prepare and distribute samples and standard reagents to members of the association and others desiring the same, to furnish blanks for tabulating analyses, and to present at the annual meeting the results of work done, discussion thereof, and recommendations of methods to be followed.

(5) The special duties of the officers of the association shall be further defined, when necessary, by the executive committee.

(6) The annual meeting of this association shall be held at such place as shall be decided by the association, and at such time as shall be decided by the executive committee, and announced at least three months before the time of meeting.

(7) No changes shall be made in the methods of analysis used in official inspection, except by unanimous consent, until an opportunity shall have been given all official chemists having charge of the particular inspection affected to test the proposed changes.

(8) Special meetings shall be called by the executive committee when in its judgment it shall be necessary, or on the written request of five members; and at any meeting, regular or special, seven enrolled members entitled to vote shall constitute a quorum for the transaction of business.

(9) The executive committee will confer with the official boards represented with reference to the payment of expenses connected with the meetings and publication of the proceedings of the association.

(10) All proposed alterations or amendments to this constitution shall be referred to a select committee of three at a regular meeting, and after report from such committee may be adopted by the approval of two-thirds of the members present entitled to vote.

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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF CHEMISTRY—BULLETIN No. 123.  
H. W. WILEY, Chief of Bureau.

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# METABOLISM OF ORGANIC AND INORGANIC PHOSPHORUS:

A FEEDING EXPERIMENT USING PHYTIN  
AND SODIUM PHOSPHATES.

BY

F. C. COOK,  
PHYSIOLOGICAL CHEMIST.



WASHINGTON:  
GOVERNMENT PRINTING OFFICE.  
1909.

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Issued June 3, 1909.

U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF CHEMISTRY--BULLETIN No. 123.  
H. W. WILEY, Chief of Bureau.

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1909.

## LETTER OF TRANSMITTAL

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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF CHEMISTRY,  
*Washington, D. C., January 15, 1909.*

SIR: I have the honor to submit for your inspection and approval a report on a phosphorus metabolism experiment conducted by F. C. Cook under the supervision of the Chief of Bureau. The report covers an experiment in rabbit feeding, extending over a period of six months, during which organic and inorganic phosphorus were fed, and includes calcium, magnesium, and total and ether-alcohol soluble phosphorus balances. At the conclusion of the experiment, complete analyses were made of the bodies of the rabbits, also of normal rabbits, which furnish some valuable data. Although the number of experiments is limited, the complete review of the literature bearing on the subject, which is included in this paper, greatly enhances its value and the interest both in this country and abroad in the relative value of the organic and inorganic forms of phosphorus, iron, etc., in the body economy makes the issuance of this contribution on the subject advisable.

I recommend that the manuscript be published as Bulletin 123 of the Bureau of Chemistry.

Respectfully,

H. W. WILEY, *Chief.*

HON. JAMES WILSON,  
*Secretary of Agriculture.*

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# METABOLISM OF ORGANIC AND INORGANIC PHOSPHORUS.

## REVIEW OF THE LITERATURE.

Much work has already been done on phosphorus metabolism, both in regard to the inorganic and organic forms of phosphorus, and many investigations have been recorded showing the advantages of the various organic forms, such as lecithin, glycerophosphoric acid, phytin, etc. Most of this work has been done abroad, although some has been published in this country, notably the researches of Jordan, Patten, and Hart;<sup>a</sup> Mendel and Underhill;<sup>b</sup> and Le Clerc and Cook.<sup>c</sup> It seemed advisable, therefore, to present a general survey of the contributions previously made on this mooted question.

## PHOSPHORUS COMPOUNDS.

In speaking of phosphorus compounds, Bunge<sup>d</sup> states that certain of them probably should be regarded as essential organic food substances for man; also that in all animal and vegetable tissues, in every cell are found two complex organic compounds which are rich in phosphorus, namely, the lecithins and the nucleins.

According to the recent recommendations of the joint committee of the American Physiological Society and the American Society of Biological Chemists on protein nomenclature, the word "proteid" should be abandoned and the word "protein" should designate that group of substances which consists essentially of combinations of  $\alpha$ -amino acids and their derivatives.

The conjugated proteins are divided into (a) nucleo-proteins, (b) glyco-proteins, (c) phospho-proteins, (d) hemoglobins, (e) lecitho-proteins. The nucleo-proteins are compounds of one or more protein molecules with a nucleic acid. The phospho-proteins are compounds of the protein molecule with some, as yet unidentified, phosphorus-containing substance other than a nucleic acid or lecithin. The lecitho-proteins are compounds of the protein molecule with lecithins (lecithans, phosphatids).

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<sup>a</sup> Amer. J. Physiol., 1906, 16 : 268.

<sup>b</sup> Ibid., 17 : 75.

<sup>c</sup> J. Biol. Chem., 1906, 2 : 203.

<sup>d</sup> Physiologic and Pathologic Chemistry, 2d ed., 1902.

## LECITHINS.

The lecithins are ester compounds which may be regarded as having been formed by the union of one molecule of glycerol with two molecules of a fatty acid (stearic acid, palmitic acid, or oleic acid), one molecule of phosphoric acid, and one molecule of cholin, with the loss of four molecules of water. The formula for lecithin is  $C_{44}H_{90}NPO_9$ . The lecithin radical contains one atom of nitrogen for every atom of phosphorus.

Cholin is an ammonium base, the composition of which is accurately known. When heated it splits into glycol (ethylene alcohol), and trimethylamin. Its synthesis corresponds with this decomposition. Wurtz <sup>a</sup> produced it by the action of ethylene oxid and water on trimethylamin. In the animal kingdom cholin has, up to the present time, been found only in lecithin. It was first obtained by Strecker <sup>b</sup> from the bile, which contains lecithin, and hence was called cholin. Liebreich <sup>c</sup> found it among the products of the decomposition of phosphorus compounds from brain tissue. Diaconow <sup>d</sup> showed that it was a product of the decomposition of lecithin. In the new tissues of plants cholin is found in other combinations as well as in lecithin. At present but little is known about the part which the lecithins play in the vital functions.

An important question is whether the lecithins of the body tissues are produced from the lecithins of the food or by synthesis from other materials such as fat, protein, and phosphoric acid. It has been ascertained from experiments on artificial pancreatic digestion that the lecithins take up water and readily split up into glycerophosphoric acid, fatty acids, and cholin. It is not yet known whether this decomposition is complete in normal digestion, or a portion is absorbed unchanged, and if so, how large a portion; whether only the undecomposed part, when absorbed, can be utilized in the building up of the tissues, or the products of decomposition which are absorbed again become united; or finally whether lecithin may also be formed from other material. The absorption of lecithin or of its products of decomposition is complete, according to Bunge, as he states that neither lecithin nor glycerophosphoric acid can be found in the feces. More recent work, however, by Long <sup>e</sup> seems to show that the feces sometimes contain lecithin in considerable quantities. The presence of lecithin in milk, eggs, and many other foods indicates that this substance is essential in nutrition.

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<sup>a</sup> Centrbl. med. Wissensch., 1868, 6 : 69, 431.

<sup>b</sup> Ann. Chem. Pharm., 1862, 123 : 353; 1868, 148 : 77.

<sup>c</sup> Ibid., 1865, 134 : 29.

<sup>d</sup> Centrbl. med. Wissensch., 1868, 6 : 97, 434.

<sup>e</sup> J. Amer. Chem. Soc., 1906, 28 : 704; Long and Johnson, *ibid.*, 1499.

## NUCLEO-PROTEINS.

By this name are designated those compound proteins which yield true nucleins on pepsin digestion and which, on cleavage with alkali, yield protein and nucleic acid. The nucleo-proteins seem to be widely distributed in the animal body. They occur chiefly in the cell nuclei, but they also often occur in the protoplasm. They may pass into the animal fluids on the destruction of the cells; hence nucleo-proteins have also been found in blood serum. They may be considered as combinations of a protein nucleus with a side chain which Kossel<sup>a</sup> calls the "prosthetic group." This side chain, which contains the phosphorus, yields on the decomposition of many nucleo-proteins, such as that from the yeast cell<sup>b</sup> or from the pancreas,<sup>c</sup> besides nucleic bases, also reducing substances, which form crystalline combinations with phenyl-hydrazin. The nucleo-proteins contain from 0.5 to 1.6 per cent of phosphorus.

The nucleo-proteins split into a nuclein and an albumin radicle and the nuclein radicle is further split into nucleic acid and albumin. The nucleic acids on cleavage yield in addition to the purin bases three simple pyrimidin derivatives, uracil, cytosin, and thymine. In a recent article by Osborne and Heyl<sup>d</sup> it appears that all but one-sixteenth of the nitrogen of nucleic acid probably belongs to guanin, adenin, cytosin, and uracil, of which one molecule of each is present for every four atoms of phosphorus.

It is important to distinguish between the nucleo-proteins and the pseudo nucleo-proteins. The latter bodies are obtained as an insoluble residue on digestion of certain nucleo-albumins or phospho-glyco-proteins with pepsin hydrochloric acid. They contain phosphorus but yield no nucleic bases. Among the pseudo nucleo-proteins may be mentioned phospho-proteins and lecitho-proteins. These substances are often fed in the form of casein or vitellin in metabolism experiments.

## NUCLEINS.

The generic name of nuclein has been bestowed upon a large number of very different organic phosphorus compounds, which are to be found in all animal and vegetable tissues, being especially abundant in the nuclei of cells. The nucleins contain about 5 per cent of phosphorus and are formed by the cleavage of nucleo-protein. The nucleins are acids, and the phosphorus is given off as phosphoric acid on boiling with water, and more rapidly on boiling with alkalis or acids. But the organic substances which are combined with the

<sup>a</sup> Arch. Anat. Physiol., Physiol. Abt., 1893, p. 157.

<sup>b</sup> Ibid., 1891, p. 359.

<sup>c</sup> Hammarsten, Zts. Physiol. Chem., 1894, 19 : 19.

<sup>d</sup> Amer. J. Physiol., 1908, 21 : 157.

phosphoric acid appear to be of varying characters. Most nucleins are protein compounds, although a few do not contain protein. Nucleins appear to occur mostly in the tissues, not in a free state, but as compounds with protein as nucleo-albumins, and perhaps also with lecithin, and the gastric digestion separates them from these bodies.

Whether the nucleins of the body tissues arise from the nucleins of food (in which case they would rank among the number of essential food substances), or whether the nucleins are formed in the body by synthesis, is a question of great importance, about which, as in the case of the mode in which the lecithins originate, very little is known. The extensive observations by Miescher <sup>a</sup> on Rhine salmon seem to show that the nucleins as well as the lecithins arise in the animal body by synthesis.

#### PHOSPHO-GLUCO-PROTEINS.

This group includes the phosphorized gluco-proteins. These compound proteins are decomposed by pepsin digestion and split off para- or pseudo-nuclein substances, similar to nucleo-albumins. They differ from the nucleo-albumins in that they yield a reducing substance on boiling with acids, and from the nucleo-proteins in that they do not yield purin bases.

Only two phosphorized gluco-proteins are known at the present time. Ichthulin, which occurs in carp eggs and was studied by Walter, <sup>b</sup> was considered by him as vitellin for a time. In regard to solubilities, ichthulin behaves like a globulin. Walter prepared a reducing substance from the para-nuclein of ichthulin, which gave a crystalline combination with phenylhydrazin. The other phospho-gluco-protein is helico-protein, obtained from the glands of the small snail *Helix pomatia*.

#### INORGANIC PHOSPHORUS.

In regard to phosphoric acid Hammarsten <sup>c</sup> states that there seems to be no doubt that its importance lies chiefly in the fact that it takes part in the formation of nucleins and thereby indirectly makes possible the processes of growth and division which are dependent upon the cell nuclei. Loew <sup>d</sup> has shown, by means of cultivation experiments on the alga *Spirogyra*, that only by supplying phosphates (in this case potassium phosphate was used) was the nutrition of the cell nucleus made possible, and thereby the growth and division of the cells. The cells of the *Spirogyra* can be kept alive, and indeed produce

<sup>a</sup> Cited in Hammarsten's Textbook of Physiological Chemistry, New York, 1908.

<sup>b</sup> Zts. physiol. Chem., 1891, 15 : 477.

<sup>c</sup> Physiological Chemistry, 2d ed., 1898.

<sup>d</sup> Biol. Centrbl., 1891, 11 : 269.



starch and proteins for some time, without a supply of phosphates, but their growth and propagation suffer. Phosphoric acid is also without doubt of importance in the formation of the lecithins and other organic phosphorus compounds. The inorganic forms of phosphorus occur in the bones and teeth as calcium phosphate and magnesium phosphate.

A small part of the phosphorus of the food is in the form of inorganic salts, as in meat, but is mostly in organic combination, as in milk, eggs, etc., as nucleo-albumin, nucleins, casein, lecithin, and vitellin.

#### PHOSPHORUS METABOLISM.

Röhmman <sup>a</sup> and his followers, Marcuse, <sup>b</sup> Steinitz, <sup>c</sup> Leipziger, <sup>d</sup> Zadik, <sup>e</sup> Ehrlich, <sup>f</sup> and Gottstein, <sup>g</sup> favor the organic forms of phosphorus, and the opinion of the majority is that the nucleins, not being easily split by the digestive juices, are absorbed with difficulty; consequently the body builds its organic compounds from the more simple organic phosphorus bodies.

The theory of Salkowski, Ueber, and the Breslau school is that the body has not the power to build from phosphorus-free protein and inorganic phosphates the organic phosphorus combinations essential to the life of the cell. Lack of phosphates in the food is without influence on phosphorus retention, and excessive feeding of organic phosphorus causes the retention of more phosphorus than the excessive feeding of inorganic phosphates. The advantages of organic phosphorus over inorganic phosphates during the period of growth is shown by Cronheim and Müller <sup>h</sup> by experiments performed on five infants and a boy. The problem was to determine whether the two forms of organic phosphorus, protein-phosphorus and fat-phosphorus, exert the same influence on the assimilation of phosphorus and nitrogen. The foods used were a casein preparation from skimmed milk and a lecithin preparation from the yolk of eggs. The food containing lecithin appeared to favor the retention of calcium, most of which was found in the bones. Experiments were also made upon five dogs, four weeks old at the beginning of the experiment. The food given consisted of milk, rice, flour, and butter. Three dogs received this diet plus egg yolk, and two received plasmon

<sup>a</sup> Berlin. klin. Wochenschr., 1898, 35 : 789.

<sup>b</sup> Arch. gesam. Physiol., 1896, 64 : 223.

<sup>c</sup> Ibid., 1898, 72 : 75.

<sup>d</sup> Ibid., 1899, 78 : 402.

<sup>e</sup> Ibid., 1899, 77 : 1.

<sup>f</sup> Stoffwechselfersuche. Inaug. Diss., Breslau, 1900.

<sup>g</sup> Ibid., 1901.

<sup>h</sup> Zts. diät. physik. Therapie, 1903, 6 : 25.

together with sodium phosphate. The amount of food given was calculated according to the following formula:  $(\sqrt[3]{\text{Body weight}})^2$ . One dog which was fed on egg yolk died, the histological section showing that death was due to pneumonia. There was no difference in the appearance of the dogs and all grew equally well. The marrow of the bones of the dogs fed on egg yolk was yellow and richer in fat, while the marrow of the bones of the plasmon-fed dogs was red and richer in blood but poorer in fat. On aging, the red marrow became yellow, proving that the dogs which were fed with egg yolk made more progress.

The same experiment was tried with four guinea pigs and one of those fed on egg yolk died of pneumonia in three months. The pigs so fed also showed fatty livers, which weighed more than the other livers. The increase in weight was greater in the case of the pigs fed on egg yolk than in the case of those which were fed plasmon. In all cases the phosphorus content of the brain was the same.

The general conclusion was that the growth of nitrogenous tissue is facilitated if phosphorus is ingested in the form of egg yolk; that is, in organic form. The daily amount of phosphorus needed by the average man, according to Siven,<sup>a</sup> is from 0.7 to 0.8 gram, and according to Ehrström,<sup>b</sup> from 1 to 2 grams. He states that phosphorus is necessary for the proper nourishment of the bones, nervous system, body proteins and cells, and that the body strives to retain the phosphates more than other salts. Other investigations along this line were carried out by Tigerstedt,<sup>c</sup> Renvall,<sup>d</sup> and Schlossmann.<sup>e</sup>

Slowtsoff,<sup>f</sup> in studying the action of lecithin on metabolism, found a plus nitrogen balance accompanied by a diminished excretion of phosphorus and also of purin bases. Where the nitrogen balance was minus, the case could be otherwise explained.

Loewi<sup>g</sup> investigated the metabolism of nucleins. He experimented on himself and found that a part of the nuclein was split in the intestine, the phosphorus of the split portion going into the feces, while the nitrogen was absorbed. The part not split was nearly all absorbed and consequently the phosphorus remained in organic combination. It is possible by nuclein feeding to bring the body into the same nitrogen and phosphoric-acid relation as exists in the nucleins themselves, since nuclein ingestion increases the retention of nitrogen and slightly increases that of the phosphorus.

<sup>a</sup> Skand. Arch. Physiol., 1901, 11 : 308.

<sup>b</sup> Ibid., 1903, 14 : 82.

<sup>c</sup> Ibid., 1904, 16 : 67.

<sup>d</sup> Ibid., 1904, 16 : 94.

<sup>e</sup> Arch. Kinderheilk., 1905, 40 : 1.

<sup>f</sup> Beitr. chem. Physiol. Path., 1906, 8 : 370.

<sup>g</sup> Arch. exper. Path. Pharm., 1900, 44 : 1; 1901, 45 : 157.

Jacob and Bergell<sup>a</sup> studied the influence of nuclein food on the blood and metabolism and found that it increases the number of the leucocytes. Brücke<sup>b</sup> conducted experiments to show that the benefit derived from egg yolk was due to lecithin. Danilewsky<sup>c</sup> determined that lecithin had great influence on the growth of young animals. Umikoff<sup>d</sup> at about the same time showed that rats and doves died when fed on a phosphorus-free diet and also when fed on an inorganic phosphorus diet plus egg albumin, and barely lived on a nuclein-phosphorus diet, but thrived when lecithin was fed. Selensky<sup>e</sup> also demonstrated the valuable effects of lecithin. Serono<sup>e</sup> was the first to inject lecithin into a human subject, and the experiment gave favorable results. Danilewsky showed that lecithin increased the number of red blood corpuscles and the hemoglobin; also that the appetite, body weight, and growth increased. Moreover, the resistance of the body to disease was greater after lecithin feeding, and the loss of body weight during hunger was decreased. Wildiers,<sup>f</sup> however, did not get results corroborating Danilewsky.

Desgrez and Zaky<sup>g</sup> experimented with guinea pigs and dogs and good results were obtained for four and one-half months after the lecithin feeding was stopped. In the urine there was more nitrogen but less phosphorus than in the controls. A larger part of the urine nitrogen was excreted as urea in lecithin-fed animals and a more complete destruction of the protein was brought about in these cases.

Gilbert and Fournier,<sup>h</sup> Carrière,<sup>i</sup> Claude and Zaky,<sup>j</sup> and others carried on clinical experiments with lecithin and found a resultant increase in appetite, number of red corpuscles, hemoblasts, and hemoglobin.

Gliken<sup>k</sup> made a study of the lecithin content of young animals born blind and helpless and of birds, eggs, etc. He states that the very young animals show a higher lecithin content than do mature animals; that the lecithin content decreases with the growth and age of the animal, and that the young animals come into the world with a large relative amount of lecithin in their bodies.

<sup>a</sup> Zts. klin. Med., 1898, 35 : 171.

<sup>b</sup> Vorlesungen über Physiologie, 2d edition, 1875, 1 : 270.

<sup>c</sup> Compt. rend., 1895, 121 : 1167.

<sup>d</sup> Biology of Phosphorus, Diss., St. Petersburg, 1895.

<sup>e</sup> Cited by Gilbert and Fournier, Compt. rend. soc. biol, 1901, 53 : 145.

<sup>f</sup> La cellule, 1900, 17 (2) : 385.

<sup>g</sup> Compt. rend., 1904, 139 : 819.

<sup>h</sup> Compt. rend. soc. biol., 1901, 53 : 145.

<sup>i</sup> Compt. rend., 1901, 133 : 314.

<sup>j</sup> Gaz. hospitaux civils militaires, 1901, No. 113, p. 1084.

<sup>k</sup> Biochem. Zts., 1907-8, 7 : 286.

Nerking<sup>a</sup> studied the lecithin distribution in animal organisms, and quotes the lecithin content of the organs of various animals as varying from 0.55 per cent in the pancreas to 1.5 per cent in the liver. Schulze<sup>b</sup> investigated the lecithin content of various plant seeds, and found from 0.5 to 1.5 per cent. This author also determined the lecithin content of various portions of the bodies of rabbits, from which it appeared that the average lecithin content equaled 0.45 per cent of the living weight of the rabbits. In the case of a hedgehog the average per cent of lecithin was 0.82 per cent of the live weight. A study of the stability of egg and brain lecithins has recently been made by Long<sup>c</sup> and a further study of lecithin emulsions was made by Long and Gephart.<sup>d</sup>

In making determinations of the deposition of lecithin and its content in organisms Franchini<sup>e</sup> found that feeding lecithin to rabbits increased the content of this substance and also of glycerophosphoric acid in the liver and the muscles, but not in the brain. Lecithin remains in the liver sometimes for fifteen days after its ingestion has been stopped. The feeding causes a slight increase of glycerophosphoric acid and of formic acid but not of cholin. Most of the ingested lecithin is absorbed, since only a very small increase is noted in the feces.

According to observations made by Merservizky,<sup>f</sup> lecithin forms 15.35 per cent of fresh hens' eggs. After six days the lecithin content diminishes. The lecithin of the yolk is a storehouse of food for the developing germ, and is used in the development of the skeletal phosphoric acid, in the building up of the phosphorus of proteins, and for the liberation of energy, after which the fat radical is oxidized.

According to Küttner,<sup>g</sup> the influence of lecithin on the activity of the digestive ferments varies with different enzymes, having a favorable effect upon the activity of the gastric and pancreatic enzymes, but a retarding effect upon others. How lecithin itself is affected he could not determine.

Koch and Reed,<sup>h</sup> in an article on the relation of the extractive to the protein phosphorus in the *Aspergillus niger*, express the view that protein, or in the case of *Aspergillus niger*, nuclein phosphorus is the most important form of phosphorus for cell life. It is formed at the expense of the other forms of phosphorus, excepting lecithin, and its formation is not diminished even in extreme starvation. In building up the nucleins lecithin probably takes no direct part. When lecithin is metabolized some or all of its phosphoric acid may be built up into nucleins as a matter of economy to the organism. The

<sup>a</sup> Biochem. Zts., 1908, 10 : 193.

<sup>b</sup> Zts. physiol. Chem., 1908, 55 : 338.

<sup>c</sup> J. Amer. Chem. Soc., 1908, 30 : 881.

<sup>d</sup> Ibid., p. 895

<sup>e</sup> Biochem. Zts., 1907, 6 : 210.

<sup>f</sup> Russky Uratch, 1907, No. 9, p. 302.

<sup>g</sup> Zts. physiol. Chem., 1906-7, 50 : 472.

<sup>h</sup> J. Biol. Chem., 1907, 3 : 49.

extractive, water-soluble forms of phosphoric acid are the ones from which the others are built and represent the intermediary steps between the phosphates and the more complex phosphorus combinations.

Kalaroukoff and Terroine<sup>a</sup> studied the influence of lecithin on the action of the pancreatic lipase and found very little, if any, increased activity when lecithin was present.

Scott,<sup>b</sup> in his experiment on phosphorus liberation from nuclein compounds, determined that it is more difficult to cause phosphorus to pass from its nucleic acid combination to an inorganic condition than has been supposed.

Michel<sup>c</sup> investigated the quality of woman's milk and found that the utilization of its nutritive materials by infants is nearly complete. The salts were least utilized, 40 per cent of calcium and 10 per cent of phosphoric acid being rejected in the feces.

Keller<sup>d</sup> studied the metabolism of phosphorus by determining the phosphoric acid in the urine of infants fed with woman's and with cow's milk, and found less phosphoric acid so excreted in the case of the breast-fed children. Whether this was due to a greater excretion in the feces or to a better assimilation of the phosphorus of the mother's milk remains to be determined.

In the experiments carried out by Jordan, Hart, and Patten,<sup>e</sup> it was found in the case of cows fed on a high phytin diet that when the amount of phytin fed was reduced the amount of fat in the milk was reduced, although there was no effect on the total solids and casein. There was also a smaller excretion of urine and a tendency to constipation. In these experiments there was a considerable loss of body phosphorus for days, when the cows were fed on a low phosphorus diet, with no apparent ill effects. The amount of phosphorus in the egesta was affected but little by the changes of the phosphorus fed; the organic phosphorus was the portion affected, if any effects were produced at all.

Some experiments by McCollum and Hart<sup>f</sup> indicate that the liver and blood have the property of cleaving the salts of phytic acid with the production of inorganic phosphoric acid. The wide distribution of inosite in the tissues makes it impossible to say as yet whether it is also a product of this cleavage. These results are in accord with those of Mendel and Underhill,<sup>g</sup> who showed that the intestine is not necessarily involved in the excretion of the metabolic products of phytin in certain animals, and also with the conclusions of Scofone,<sup>h</sup> that the enzymes of the digestive tract do not alter phytin. Exami-

<sup>a</sup> Compt. rend. soc. biol., 1907, 63: 372.

<sup>b</sup> Brit. Med. J., 1906, (2) p. 1791.

<sup>c</sup> L'obstetrique, Paris, 1897, 2: 518.

<sup>d</sup> Abs., Chem. Centrbl., 1899, 70: 54.

<sup>e</sup> Amer. J. Physiol., 1906, 16: 268.

<sup>f</sup> J. Biol. Chem., 1908, 4: 497.

<sup>g</sup> Amer. J. Physiol., 1906, 17: 75.

<sup>h</sup> Abs., Biochem. Centrbl., 1905, 3: 606.

nations of the action of ptyalin, pepsin, and trypsin have confirmed Scofone's results.

Experiments made with extracts of muscle and kidney did not give results which pointed toward the presence of a phytase in these tissues.

Suzuki and Yoshimura<sup>a</sup> studied the distribution of anhydroxy-methylene-phosphorus (phytin), and give a method for extracting the compound, which is a calcium or a magnesium salt. In the juice of tubers and fruit more inorganic than organic phosphorus is found.

Suzuki, Yoshimura, and Takaishi<sup>b</sup> made an investigation of the enzym which decomposes anhydroxymethylene diphosphoric acid, and state that when rice bran and water are allowed to stand the organic compound will be decomposed and the amount of soluble inorganic phosphoric acid increased. When boiled this action does not take place. The same change takes place when barley and rape seeds are used. No other enzym will do this.

As opposed to the beneficial results of organic phosphorus Keller<sup>c</sup> got very favorable results from feeding normal milk plus inorganic phosphates.

Kochmann<sup>d</sup> studied the changes in the inorganic constituents in the tissues of rabbits poisoned by phosphorus. He made iron, calcium, magnesium, phosphorus, potassium, and sodium estimations in the liver, heart, muscles, and bones and compared them with similar estimations in normal animals. His conclusions are that a definite effect was produced on phosphorus metabolism and that the use of phosphorus in bone affections and as a stimulant is well founded. Calcium, potassium, and sodium replace one another. The magnesium metabolism is also affected in the cases of phosphorus poisoning, and the excretion of phosphorus and calcium run parallel.

More recent work by Hart and McCollum<sup>e</sup> on feeding inorganic phosphates to growing pigs has been conducted for two years. According to the abstract published by the authors, the results clearly indicate that inorganic phosphates, such as bone ash, finely ground rock phosphate, or precipitated calcium phosphate (a mixture of di- and tri-calcium phosphates) can be used by these animals in connection with rations containing insufficient phosphorus. Young animals of 40 pounds weight, receiving inorganic phosphates, together with other salts as supplementary to a ration very low in mineral constituents, grew to be animals of 280 pounds weight,

<sup>a</sup> Abs., Chem. Centrbl., 1907, 78:1636.

<sup>b</sup> Ibid., 1637.

<sup>c</sup> Abs., Zts. diät. physik. Therapie, 1901, 5:147.

<sup>d</sup> Arch. gesam. Physiol., 1907, 119:417.

<sup>e</sup> Abs., Science, 1908, 28:217.

and bore litters of fairly vigorous pigs, which on the same ration completed the cycle back to 80 pounds, while animals on the same ration, without the inorganic phosphates, collapsed in three months, losing weight and the use of their legs. Other important observations made are as follows: (1) Animals on a ration extremely low in phosphorus made as large gains, up to 75 to 100 pounds, as did animals receiving an abundance of this element, but after reaching this point the weight was reduced and collapse followed. (2) When such low phosphorus rations as induced these symptoms were supplemented by inorganic phosphates, no unfavorable results appeared. Animals fed a low phosphorus diet, supplemented by inorganic phosphates, made as vigorous a development as other animals receiving all the phosphorus in the organic form. (3) Determination of calcium and phosphorus in the principal organs and tissues of the animals fed on the low phosphorus ration showed that they maintained their normal body composition. The per cent of ash in the skeleton of pigs on a depleted phosphorus ration was reduced to nearly one-half that of pigs which received a normal ration, or the phosphorus-poor ration plus inorganic phosphates. When the animals were starving for phosphorus they derived it from their bones, but always removed calcium and phosphorus in the proportions found in tricalcium phosphate.

#### PHOSPHORUS ELIMINATION.

In studying the absorption and elimination of phosphorus and other substances the influence of the reactions of the gastro-intestinal tract is of great importance, as is the character of the ash of the food ingested. All of the conditions influencing acidity and alkalinity affect the absorption and the path of excretion of phosphorus, calcium, and magnesium salts. In the case of herbivora a large portion of these elements is eliminated in the feces, no absorption having taken place. This is likely to happen when the food ash is alkaline and sufficient calcium and magnesium are present to combine with the phosphoric acid. There is then an excretion of these elements through the intestines as well as through the kidneys, and when alkalis are in excess of acids in the gastro-intestinal tract the elimination through the bowel is likely to exceed that through the urine. In omnivorous animals a larger portion of the phosphorus, calcium, and magnesium, as well as the nitrogen, is eliminated by the kidneys than is the case with herbivorous animals.

The subject of phosphorus elimination has been studied under many pathological conditions, and especially in hunger, in the cases of Cetti,<sup>a</sup> Breihaupt,<sup>b</sup> and others.

<sup>a</sup> Senator and Müller: *Virchow's Archiv. Suppl.*, 1893, 131 : 2.

<sup>b</sup> Müller: *Virchow's Archiv, Suppl.*, 1893, 131 : 52.

A phosphoric-acid diabetes, noted by Teissier,<sup>a</sup> and Ralfe,<sup>b</sup> showed a resulting polyurea where as much as 12 grams of phosphoric acid were eliminated per day by the kidneys. In diseases of the kidneys the activities of these organs in eliminating the phosphates may be considerably diminished. In meningitis, on the contrary, a marked increase in the phosphates eliminated is observed in the urine. The statements in regard to the quantity of phosphates in the urine in rachitis and in osteomalacia are somewhat contradictory. A phosphaturia is described, which is more correctly called an alkalinuria, where the phosphates settle out owing to an alkaline reaction. A pathological phosphaturia is also noted. Sendtner<sup>c</sup> showed that there was an increased calcium excretion in cases of phosphaturia. This condition is due to a perversion of metabolism, but serves to illustrate the close relationship which exists between calcium and phosphoric acid.

Voit<sup>d</sup> found that the feces of starving dogs contained phosphates.

The subject of phosphorus elimination has been quite fully investigated by Paton, Dunlop, and Aitchison.<sup>e</sup> In the case of dogs fed on a vegetable diet a large proportion of the phosphorus of the food is not eliminated in the urine. The same thing is true when the phosphorus (inorganic) is injected subcutaneously.

In the case of goats none of the subcutaneously injected phosphorus is found in the urine, neither is any of the body or food phosphorus found in the urine. During lactation the excretion of phosphorus by the bowel is diminished to meet the requirements of milk formation. In the case of dogs there is a diminished excretion of phosphorus in the urine during lactation. The milk of goats contains a large amount of total phosphorus, but a small percentage of organic combined phosphorus.

On giving a soluble glycerophosphate of calcium by the mouth no increased excretion of phosphorus was detected in the urine of dogs or in the urine or milk of goats.

The excretion of inorganic constituents in the urine was studied by Cathcart and Fawsitt<sup>f</sup> during a fourteen-day fasting period. The excretion of phosphorus fell off gradually. There was a decreased output of calcium, magnesium, sodium, and potassium. The normal ratio of sodium and potassium is reversed in starvation.

Fitz, Alsberg, and Henderson's<sup>g</sup> determinations of phosphoric acid excretion during experimental acidosis in rabbits are to the

<sup>a</sup> Lyon Médical, 1875, 19:307.

<sup>b</sup> Lancet, 1887 (2), p. 1243.

<sup>c</sup> Münch. med. Wochenschr., 1888, 35:671.

<sup>d</sup> Hermann's Handbuch der Physiologie, 1881, 6:345.

<sup>e</sup> J. Physiol., 1900, 25:212.

<sup>f</sup> Ibid., 1907, 36:27.

<sup>g</sup> Amer. J. Physiol., 1907, 18:113.



effect that feeding hydrochloric acid produces first an increase and then a decrease in the phosphorus ( $P_2O_5$ ) excreted in the urine. The determination favors the view that the body phosphates are concerned with neutralizing the acid and with its removal from the body.

Roos<sup>a</sup> on feeding thyroids to dogs got an increased phosphoric acid excretion and after extirpation of the thyroids found that the elimination of phosphorus was decreased. Phosphorus elimination seems to be regulated, in part at least, by those glands, the relationship being similar to that which probably exists between calcium and phosphoric acid and the ovaries, and that between iron and the spleen.

It is difficult to give a typical urine analysis on account of its variations. The following table may be of some value, though only approximate figures are given for the quantities of the most important inorganic constituents which are eliminated by an average-sized person on a mixed diet in the course of twenty-four hours in a quantity of 1,500 cc:

	Grams.
Sodium chlorid ( $NaCl$ ) .....	15.0
Sulphuric acid ( $H_2SO_4$ ) .....	2.5
Phosphoric acid ( $P_2O_5$ ) .....	2.5
Potash ( $K_2O$ ) .....	3.3
Ammonia ( $NH_3$ ) .....	.7
Magnesia ( $MgO$ ) .....	.5
Lime ( $CaO$ ) .....	.3
Remaining inorganic bodies .....	.2
Total .....	25.0

Phosphoric acid occurs in acid urines partly as double  $MH_2PO_4$ , and partly as simple  $M_2HPO_4$ , both of these phosphates being found in acid urines at the same time. Ott<sup>b</sup> found that on an average 60 per cent of the total phosphoric acid was double, and 40 per cent was simple acid phosphate. The total quantity of phosphoric acid is variable and depends on the kind and the quantity of the food. The average quantity of phosphoric acid eliminated by man is in round numbers 2.5 grams, with a variation of from 1 to 5 grams per twenty-four hours. A small part of the phosphoric acid of the urine originates from the burning of organic compounds such as nuclein, protagan, and lecithin within the organism. The greater part originates from the phosphates of the food, and the quantity of eliminated phosphoric acid is greater when the food is rich in alkali phosphates in proportion to the quantity of lime and magnesium phosphates. If the food contains much lime and magnesium, large quantities of earthy phosphates are eliminated in the excrements; and even though the food contains considerable amounts of phosphoric acid in

<sup>a</sup> Zts. physiol. Chem., 1895-6, 21: 19.

<sup>b</sup> Zts. physiol. Chem., 1886, 10: 1.

these cases, the quantity of phosphoric acid in the urine is small. Such a condition is found in herbivora, whose urine is habitually poor in phosphates. The extent of the elimination of phosphoric acid by the urine depends not only upon the total quantity of phosphorus in the food, but also on the relative amounts of alkaline earths and the alkali salts in the food. According to Preysz<sup>a</sup> and Klug<sup>b</sup> and Olsavszky, the elimination of phosphoric acid is considerably increased by intense muscular work.

From the transformation of tissues rich in protein or phosphorized nerve substances in the body, an equal relation between the nitrogen and the phosphoric acid in the urine might be expected. Many investigations have been made on this point, but the conditions which affect the elimination of phosphoric acid are not yet sufficiently known to permit any definite conclusions being drawn from the observations thus far made.

Of the various forms of phosphate compounds which appear in the urine the following may be mentioned: Tricalcium phosphate,  $\text{Ca}_3(\text{PO}_4)_2$ , which occurs only in alkaline urines; calcium diphosphate ( $\text{CaHPO}_4 + 2\text{H}_2\text{O}$ ) occurs in neutral or only in very faintly acid urines; ammonium magnesium phosphate, triple phosphate, may separate, of course, from an amphoteric urine in the presence of a sufficient quantity of ammonium salts, but it is generally characteristic of a urine which has become ammoniacal through alkaline fermentation; amorphous magnesium triphosphate,  $\text{Mg}_2(\text{PO}_4)_2$ , occurs with calcium triphosphate in urine rendered alkaline by a fixed alkali, and crystalline magnesium phosphate ( $\text{Mg}_2(\text{PO}_4)_2 + 22 \text{H}_2\text{O}$ ) which has been observed in a few cases in human urine, and in horses' urine.

Phosphate calculi may consist of a mixture of the normal phosphate of alkaline earths with triple phosphate. They also are composed of a mixture of earthy phosphate, triple phosphate, and ammonium urate, surrounding a foreign body as a nucleus. Calculi consisting of triple phosphates alone and stones of simple acid calcium phosphate are seldom obtained.

#### SALTS IN THE ORGANISM.

The body contains in its tissues and liquids a considerable amount of inorganic material. When any organ is incinerated this material remains as ash. If the bones, which are rich in mineral material, are omitted the average amount of ash in the human body amounts to about 0.1 per cent of its weight. It consists of chlorids, phosphates, sulphates, carbonates, fluorids, silicates of potassium, sodium, calcium,

<sup>a</sup> Maly's Jahres-Ber., 1891, 21:352.

<sup>b</sup> Arch. gesam. Physiol., 1893, 54:21.

magnesium, and iron; iodine occurs also, especially in the thyroid tissues. In the liquids of the body the main salts are sodium chlorid, sodium carbonate, sodium phosphate, and potassium and calcium chlorid or phosphate. In considering the organic foodstuffs, their value as sources of energy, as well as their function in constructing tissue, is emphasized. The salts have no importance from the former point of view. Whatever chemical changes they undergo are not attended by the liberation of heat energy—none at least of sufficient importance to be considered. They have, however, most important functions, as they maintain a normal composition and osmotic pressure in the liquids and tissues of the body, and by virtue of their osmotic pressure play an important part in controlling the flow of water to and from the tissues. Moreover, these salts constitute an essential part of the composition of living matter. In some way they are bound up in the structure of the living molecule and are necessary to its normal reactions or irritability. Even the proteins of the body liquids contain definite amounts of ash, and if this ash is removed their properties are seriously altered, as is shown by the fact that native proteins when made practically ash-free lose their property of coagulation by heat. The globulins are precipitated from their solutions when the salts are removed. The special importance of the calcium salts in the coagulation of the blood and the curdling of milk is well known, as is also the peculiar part played by the calcium, potassium, and sodium salts in the rhythmical contractions of the heart muscle and the irritability of muscular and nervous tissues. The special importance of the iron salts for the production of hemoglobin is also well known. The nutritive importance of the salts in the diet has been demonstrated by direct experiment.

Dogs were fed by Forster<sup>a</sup> upon a diet composed of ash-free fats and carbohydrates, and meats which had been extracted with water until the salts had been reduced. The animals were in a dying condition at the end of twenty-six to thirty-six days. It is probable that they would have lived longer if deprived of food entirely, with the exception of water, since the metabolism of the abundant diet provided aided in increasing the loss of salts from the body. Lunin<sup>b</sup> has described experiments which indicate that some at least of our salts must be provided for us in organic combinations such as are found in plant and animal foods. In his experiments he found that mice fared well on a diet of dried cow's milk. If fed, however, on a diet containing the organic but ash-free constituents of milk, namely, sugar, fat, and casein, together with the extracted salts of cow's milk, they died in from twenty to thirty days.

<sup>a</sup> Arch. Hygiene, 1884, 2:385.

<sup>b</sup> Zts. physiol. Chem., 1881, 5:31.

In a recent article on alkali salts in the ash of human and cow's milk, Kastle<sup>a</sup> states that the practices which have for their object the reduction of the amount of fat in cow's milk, or the addition thereto of mineral matter available for neutralizing the acids resulting from the processes of metabolism are based on sound practical experience, the important difference between the two kinds of milk as to mineral constituents being as follows: (1) Human milk contains relatively more of its mineral matter in utilizable form than cow's milk; (2) it can supply the organism of the child with relatively larger amounts of available alkali in proportion to the protein than cow's milk.

The idea is advanced that in the milk of various animals the inorganic constituents are present in the same proportion as in the ash of the young animals. Bunge<sup>b</sup> shows that there is a very close relation between the composition of the ash of young rabbits, dogs, and cats, and that of dog's milk, dog's blood, and dog's blood serum. He also makes the statement that the epithelial cells of the mammary glands select from the blood and give to the milk all the inorganic constituents in the proportion needed by the young animal.

Phosphoric acid is an important constituent of milk. According to the same author woman's milk contains 0.31 to 0.45 gram of phosphoric acid per liter, and cow's milk 1.81 to 1.97 grams. It is an important fact that the food of the young furnishes the phosphoric acid in organic combinations.

Many investigators have shown that the phosphorus of cow's milk is not so well absorbed as that of woman's milk. Stoklasa<sup>c</sup> claims that the lecithin phosphorus content of woman's milk is 0.35 per cent, as compared with 0.5 per cent in cow's milk. Blauberg<sup>d</sup> fed a child on mother's milk and studied the metabolism of the salts contained therein. He compared his results with the results obtained by other investigators and concluded that the constituents of mother's milk seem to be better utilized by the system than the constituents of cow's milk.

No complete analyses of the mineral substances of pure, blood-free muscle substance were found. The ash remaining after burning the muscle (which amounts to about 10 to 15 parts per thousand, calculated on the moist muscle) is acid in reaction. The chief mineral constituents are potassium and phosphoric acid. Next in amount are sodium and magnesium, and lastly calcium, chlorine, and iron oxid. Sulphates only exist as traces in the muscles, but are formed by the burning of the proteins, and, therefore, occur in abundant quantities in the ash. The muscles contain such large quantities of potassium and

<sup>a</sup> Amer. J. Physiol., 1908, 22 : 284.

<sup>b</sup> Zts. Biol., 1874, 10 : 111, 295.

<sup>c</sup> Zts. physiol. Chem., 1895-6, 21 : 79.

<sup>d</sup> Abs., Chem. Centrbl., 1897, 68 : 957.

phosphoric acid that potassium phosphate seems to be unquestionably the predominating salt. Chlorin is found in such insignificant quantities that it is perhaps derived from a contamination with blood or lymph. The quantity of magnesium is about double that of calcium. These two bodies, as well as iron, occur only in very small amounts.

Sherman<sup>a</sup> in making a determination of the amount of mineral matter required by the human body, examined twenty American dietaries for ash and compared the amount of mineral matter contained in them with the estimated maintenance requirements as found in metabolism experiments. He concludes that iron and protein run parallel and that calcium and phosphoric acid vary; further that the average diets do not supply a sufficient amount of either calcium or phosphoric acid, and as much attention should be paid to the supply of calcium, phosphoric acid, and iron as to protein. Milk and cheese might be substituted for a part of the meat of the ordinary diet and the use of fruits and vegetables should supply a part of the sugar, starch, and minerals. Several other workers have studied ash-free diets.

#### CALCIUM COMPOUNDS.

The metabolism of calcium has been extensively studied. There are two forms of calcium which enter into the composition of our food and drink, the organic form in milk, eggs, plant seeds, etc., and the inorganic form, which consists principally of calcium carbonate, calcium sulphate, and calcium phosphate. Both forms are absorbable, the amount absorbed depending on the food taken simultaneously. Among other factors influencing the calcium absorption may be mentioned sodium chlorid, which increases, and the alkalis, which diminish, the amount of calcium absorbed. As noted before, there exists a close relationship between calcium and phosphoric acid.

According to Bunge<sup>b</sup> and Bertram<sup>c</sup> the calcium in plant food is not so well absorbed as that in animal food. Many foods lack calcium, the daily need of which for the human body is, in the opinion of Oberndörffer,<sup>d</sup> 1.5 grams, while Bunge<sup>e</sup> claims double that amount, or 3 grams per diem. The young need milk rich in calcium. Bunge says calcium forms 0.04 per cent of the blood, while Albu and Neuberg<sup>f</sup> cite experiments showing that calcium forms as much as 0.27 per cent of the blood. In arterio sclerosis, Gazert<sup>g</sup>

<sup>a</sup> Lake Placid Conf., Home Econ. Proc., 1907, 9 : 114.

<sup>b</sup> Lehrbuch der Physiologie des Menschen, 1901, 2 : 88.

<sup>c</sup> Abs., Chem. Centrbl., 1879, 10 : 526.

<sup>d</sup> Berlin. klin. Wochenschr., 1904, 41 : 1068.

<sup>e</sup> Zts. Biol., 1876, 12 : 191.

<sup>f</sup> Mineral Stoffwechsel, Berlin, 1906.

<sup>g</sup> Deutsch. Arch. klin. Med., 1898, 62 : 390.

found from fifteen to twenty times as much calcium in the blood as in normal health. More calcium is absorbed from natural food than from artificial, and in the latter case more calcium is likely to be excreted by the bowels unaltered than normally. The amount of calcium retained by the tissues and its manner of combination depend both upon the quality of the food and the amount of calcium in it.

With the exception of the importance of the alkaline earths as carbonates, and especially as phosphates, on the physical composition of certain structures, such as the bones and teeth, their physiological importance is nearly unknown. The occurrence of earthy phosphates in all proteins, and their great importance in the passage of the proteins from a soluble to a coagulable state, make it probable that the earthy phosphates play an important part in the organization of the proteins. An insufficient supply of alkali earths in the food raises an interesting question as to the effect of this lack on the bony structure.

#### CALCIUM SALTS AND COAGULATION.

The property which is the most characteristic of casein is that it coagulates with rennet in the presence of a sufficiently great amount of lime salts. In solutions free from lime salts the casein does not coagulate with rennet, but if lime salts are added it is changed so that the solution (even if the enzym is destroyed by heating) yields a coagulated mass, having the properties of curd.

According to Soxhlet<sup>a</sup> the soluble lime salts are only of essential importance in coagulation, while the calcium phosphate is without importance. According to Courant<sup>b</sup> the calcium casein compound on coagulation may carry down with it, if the solution contains dicalcium phosphate, a part of this as tricalcium phosphate, leaving monocalcium phosphate in the solution. The chemical process which takes place in the rennet coagulation has not been thoroughly investigated.

The fibrin ferment, which was called thrombin by Schmidt,<sup>c</sup> is produced, according to Pekelharing,<sup>d</sup> by the action of soluble calcium salts on a preformed zymogen existing in the noncoagulated plasma. Schmidt admits the presence of such a mother-substance of fibrin ferment in the blood and calls it prothrombin.

Brücke<sup>e</sup> showed long ago that fibrin left an ash containing calcium phosphate. The fact that calcium salts may facilitate or even cause a coagulation in liquids poor in fibrin ferment has been known for a

<sup>a</sup> Münch. med. Wochenschr., 1893, 40 : 61.

<sup>b</sup> Arch. gesam. Physiol., 1891, 50 : 109.

<sup>c</sup> Zur Blutlehre, Leipzig, 1892.

<sup>d</sup> Zts. physiol. Chem., 1896-7, 22 : 245.

<sup>e</sup> Vorlesungen über Physiologie, 2nd ed., 1875, 1 : 270.

number of years through the researches of Green,<sup>a</sup> Ringer and Sainsbury,<sup>b</sup> and others. The necessity of the lime salts for coagulation was first shown positively by the important investigations of Arthus and Pagès.<sup>c</sup> In regard to the manner in which the lime salts act a conclusion has been reached by Freund,<sup>d</sup> who claims that the separation of the excess of calcium phosphate is the cause of a part of the protein becoming insoluble—that is, a cause for coagulation. Weighty objections to this view can be raised, and it is refuted by Latschenberger<sup>e</sup> and Strauch.<sup>f</sup> According to Pekelharing<sup>g</sup> the process is as follows: The prothrombin is converted into thrombin by the action of the soluble lime salts, and fluids which are in all other respects capable of coagulation, but contain only prothrombin and no thrombin, can therefore be coagulated by the addition of soluble lime salts. Thrombin is a lime combination of prothrombin, and the process of coagulation consists in the thrombin carrying the lime to the fibrinogen, which is converted into the insoluble combination of fibrin and lime. Several important papers have appeared, notably those of Field,<sup>h</sup> Morawitz,<sup>i</sup> and Loeb,<sup>j</sup> dealing with the rôle of calcium in the coagulation of the blood. While the literature on this subject has not been fully covered in this report, its importance demands more than a passing reference in a paper dealing with calcium metabolism. It has been shown by the investigations of Cavazzani<sup>k</sup> that the lime salts are of importance in the coagulation of the muscle-plasma as well as in that of the blood.

The inorganic constituents of the bony structure, the so-called bone earths, which remain after the complete calcination of the organic substance as a white, brittle mass, consist chiefly of calcium and phosphoric acid, but also contain carbon dioxide and, in smaller amounts, magnesium, chlorine, and fluorine. Alkali sulphates and iron, which have been found in bone ash, do not seem to belong to the bone tissue itself, but to the nutritive fluid or other parts of the bones. According to Gabriel<sup>l</sup> potassium and sodium are essential constituents of bone ash. The opinions of investigators differ somewhat as to the manner

<sup>a</sup> *J. Physiol.*, 1887, 8 : 372.

<sup>b</sup> *Ibid.*, 1890, 11 : 369.

<sup>c</sup> *Abs.*, *Chem. Centrbl.*, 1891 (1), p. 511.

<sup>d</sup> *Ibid.*, 1889 (1), p. 545.

<sup>e</sup> *Abs.*, *Chem. Centrbl.* 1890 (1), p. 169.

<sup>f</sup> *Blutgerinnungstheorie*. Diss., Dorpat, 1889.

<sup>g</sup> *Abs.* *Chem. Centrbl.*, 1892 (2), p. 335.

<sup>h</sup> *Centrbl. Physiol.*, 1903, 17 : 529.

<sup>i</sup> *Deut. Arch. klin. Med.*, 1903-4, 479 : 1.

<sup>j</sup> *Beitr. chem. Physiol. Path.*, 1903-4, 5 : 191; 1904-5, 6 : 260; *Arch. Path. Anat. Physiol.*, 1903, 173 : 35; 1906, 185 : 160; *J. Med. Research*, 1903, 10 : 407.

<sup>k</sup> *Maly's Jahres-Ber.*, 1892, 22 : 346.

<sup>l</sup> *Zts. physiol. Chem.*, 1893-4, 18 : 257.

in which the mineral bodies of the bony structure are combined with each other. Chlorin and fluorin are present in the same form as in apatite ( $\text{CaFl}_2, 3\text{Ca}_3\text{P}_2\text{O}_8$ ). If the magnesium, chlorin, and fluorin be eliminated, the last according to Gabriel occurring only as traces, the remaining mineral bodies form the combination  $3(\text{Ca}_3\text{P}_2\text{O}_8)\text{CaCO}_3$ . According to this author the simplest expression for the composition of the ash of the teeth is  $(\text{Ca}_2(\text{PO}_4)_2 + \text{Ca}_5\text{H}_4\text{P}_3\text{O}_{13} + \text{H}_2\text{O})$ , in which 2 to 3 per cent of the lime is replaced by magnesia, potash, and soda, and 4 to 6 per cent of the phosphoric acid by carbon dioxid, chlorin, and fluorin. Analyses of bone earths have shown that the mineral constituents exist in rather constant proportions, which is nearly the same in different animals. The diverse quantitative composition of the various bones of the skeleton depends probably on the varying quantities of other formations, such as the marrow, blood vessels, etc., which they contain. This probably also explains the larger quantity of organic substance in the spongy parts of the bones as compared with the more compact parts. Schrodt<sup>a</sup> has made comparative analyses of different parts of the skeleton of the same animal (dog), and has found an essential difference. The quantity of water in the fresh bones varies from 138 to 438 parts per thousand. The composition of bones at different ages has not been definitely determined, but according to the analyses made by Voit<sup>b</sup> of bones of dogs and by Brubacher<sup>c</sup> of the bones of children it appears that the skeleton becomes poorer in water and richer in ash with increase in age. Grafenberger<sup>d</sup> has found that the bones of rabbits from  $6\frac{1}{2}$  to  $7\frac{1}{2}$  years old contained only 14 to 17 per cent of water, while the bones of full grown rabbits from 2 to 4 years old contained 20 to 24 per cent. The bones of old rabbits contain more carbon dioxid and less calcium phosphate than do those of young ones.

#### CALCIUM METABOLISM.

A great many experiments have been made to determine the change in the bone constituents, for instance, when a ration rich in lime and one deficient in lime is fed, but the results have always been indecisive or contradictory. The attempts to substitute other alkaline earths or clay for the lime of the bones have also given unsatisfactory results. Weiske<sup>e</sup> has shown that when young and still rapidly growing rabbits are fed with oats, which are poor in acid and lime, plus magnesium and strontium carbonate, these substances in part pass into the skeleton, but a physiological replacement of lime by magnesium or strontium is not to be expected. On the administration of

<sup>a</sup> Maly's Jahres-Ber., 1877, 6:207.

<sup>d</sup> Maly's Jahres-Ber., 1891, 21:290.

<sup>b</sup> Zts. Biol., 1880, 16:55.

<sup>e</sup> Abs., Chem. Centrbl., 1892 (2), p. 590.

<sup>c</sup> Ibid., 1890, 27:517.



madder the bones of the animal are found to be colored red after a few days or weeks; but these experiments have not led to any positive conclusion in regard to the growth or metabolism of the bones.

Under pathological conditions, as rachitis and softening of the bones, an ossein has been found which does not give any typical gelatin on boiling with water. Otherwise pathological conditions seem to affect chiefly the quantitative composition of the bones, and especially the relationship between the organic and inorganic substances. Attempts have been made to produce rachitis in animals by the use of foods deficient in lime. From experiments on fully developed animals contradictory results have been obtained. In young, undeveloped animals Voit<sup>a</sup> produced, by lack of lime salts in the food, a change similar to rachitis. In full-grown animals the bones were changed after a long time because of the lack of the lime salts in the food, but did not become soft, only thinner (osteoporosis). The experiments in which the lime salts were removed from the bones by the addition of lactic acid to the food have led to no positive results (Heitzmann,<sup>b</sup> Heiss,<sup>c</sup> Baginsky<sup>d</sup>). Weiske<sup>e</sup> on the contrary, has shown by administering dilute sulphuric acid or monosodium phosphate with the food (presupposing that the food gave no alkaline ash) to sheep and rabbits, that the quantity of mineral matter in the bones might be diminished. A few investigators are of the opinion that in rachitis, as in osteomalacia, a solution of the lime salts by means of lactic acid takes place. This was suggested by the fact that Weber and Schmidt<sup>f</sup> found lactic acid in the cyst-like altered bony substance in osteomalacia. Well-known investigators have disputed the possibility of the lime salts being washed from the bones in osteomalacosis by means of lactic acid. The recent investigations of Levy<sup>g</sup> contradict the statement as to the solution of lime salts by lactic acid in osteomalacia. He has found that the normal relationship  $6P_2O_5:10Ca$  is retained in all parts of the bones in osteomalacia, which would not be the case if the bone earths were dissolved by an acid. The decrease in phosphates occurs in the same quantitative relationship as the carbonate; and, according to Levy, in osteomalacia the exhaustion of the bone takes place by decalcification, in which one molecule of phosphate and calcium after the other is removed.

<sup>a</sup> Zts. Biol., 1880, 16 : 55.

<sup>b</sup> Maly's Jahres-Ber., 1873, 3 : 229.

<sup>c</sup> Zts. Biol., 1876, 12 : 151.

<sup>d</sup> Virchow's Archiv, 1882, 87 : 301.

<sup>e</sup> Abs., Chem. Centrbl., 1892 (2), p. 590.

<sup>f</sup> Cited from Gorup-Besanez. Lehrbuch der physiologischen Chemie, 3d Edition, Braunschweig, 1874, p. 636.

<sup>g</sup> Zts. physiol. Chem., 1894, 19 : 239.

The relative amounts of calcium and phosphoric acid in the teeth are, according to the analysis of Hoppe-Seyler,<sup>a</sup> about the same as in bone earths.

The importance of calcium for the activity of the nervous system and the muscles has been the subject of study by many investigators. The conclusions drawn are that if the amount of calcium is decreased nervous and muscular irritability will result and, conversely, that an increase of the calcium will diminish the irritability of the nerves and muscles. Ringer proved that the frog's heart can be kept beating for long periods upon a mixture of sodium chlorid, potassium chlorid, and calcium phosphate or chlorid, and he laid especial importance upon the calcium. The calcium ions are present in relatively small quantities in the blood, but they are absolutely necessary to contractility and irritability. When present in quantities above normal or when in proportional excess over the sodium or potassium ions they cause a condition of tonic contraction that has been designated as calcium rigor. The calcium promotes a state of contraction, the sodium and the potassium a state of relaxation.

Tigerstedt in his text-book states that calcium salts favor the contraction of the heart, while potassium salts are important for its relaxation. Calcium favors muscular movements of low forms of animal life—the contractility of both skeletal and smooth muscles. He cites the experiments of Voit,<sup>b</sup> who fed pigeons with food containing no calcium, and found that the bones which were used for movements were normal for calcium, while the sternum and skull bones were brittle and even perforated in places.

Falta and Whitney<sup>c</sup> showed that after extirpation of a dog's pancreas, the calcium elimination was increased, though the nitrogen, phosphoric acid ratio remained unchanged. The excretion of uric acid in these cases was doubled.

The importance of calcium salts for the growing organisms is discussed by Aron and Sebauer.<sup>d</sup> Special attention was given to the calcium content of the bones, brains, nerves, muscles, and blood. Dogs and rabbits were used, half of them being fed on a calcium-poor diet. The young animal requires at least 1.2 per cent of its body weight of calcium; a diet supplying a smaller amount is called a calcium-poor diet. Under such conditions nervous and other disorders follow, a condition like rickets being established after continued feeding of such a diet; in these cases the bones contain more water than is normal, that is, a water-rich bone is developed whose

<sup>a</sup> Hammarsten, Textbook of Physiological Chemistry, New York, 1908, p. 440.

<sup>b</sup> Zts. Biol., 1880, 16 : 55.

<sup>c</sup> Beitr. chem. Physiol. Path., 1908, 11 : 224.

<sup>d</sup> Biochem. Zts., 1908, 8 : 1.

organic framework is poor in calcium. The calcium content of the flesh and blood shows no variation and the brain but a slight variation from the normal.

Following some experiments made by Sanford and Lusk at the Yale Medical School on new-born pigs, Wilson<sup>a</sup> studied the influence of diet on the growth of young pigs. Three pigs were killed and analyzed at birth and three were reared on a skim-milk diet. To the diet of one pig, lactose was added; to that of the second, dextrose; and the third was given the skim milk without any added substance. The lactose-fed pig thrived best, while the pig fed on skim milk alone showed the least progress after sixteen days. The analyses showed that the pig fed on skim milk used 52 per cent of the calcium in the food for growth; the lactose-fed pig used 70 per cent; and the dextrose-fed pig 64 per cent. The calcium content of the bodies of the pigs at the end of the experiment was 8.29, 8.03, and 8.13 per cent, respectively. Calcium storage evidently depends on the development of the animal rather than on any specific influence of the milk constituents. Herter<sup>b</sup> found striking retardations in the development of the skeleton of older pigs fed on skim milk for many months, but no evidence of rickets was seen.

W. Camerer, jr.,<sup>c</sup> finds that the calcium content of mothers' milk is barely sufficient to cover the needs of the nursing infant if the percentage composition of the five-months-old baby were the same as that of the new-born baby. The percentage of calcium in the new-born pigs above noted averages 9.4 per cent at birth and is 8.15 per cent at the end of two and one-half weeks' feeding. If the pigs fed on lactose and dextrose had contained 9.4 per cent calcium at the end of the two and one-half weeks' test, an almost complete calcium absorption would have taken place.

Patterson<sup>d</sup> states that when an animal is deprived of all inorganic salts in its food profound constitutional disturbances, resulting in death, are produced. The salts of the blood must not only be present in sufficient quantity to bring the osmotic pressure of the blood to a constant value, but they must also be present in certain definite ratios. Every living cell of the body must be washed by a fluid containing salts of certain monovalent and divalent metals in an unvarying ratio, otherwise a disturbance in the intracellular ion-proteins (Loeb)<sup>e</sup> or colloidal salts (Osborne)<sup>f</sup> is produced. Bearing in mind this necessity for a constant ratio between the various salts of the blood, a number of interesting questions are raised by Patterson in regard to the probable effects of depriving an animal, com-

<sup>a</sup> Amer. J. Physiol., 1902, 8 : 197.

<sup>b</sup> J. Exper. Med., 1898, 3 : 293.

<sup>c</sup> Zts. Biol., 1902, 43 : 1.

<sup>d</sup> Bio-Chem. J., 1908, 3 : 39.

<sup>e</sup> Dynamics of Living Matter, New York, 1906.

<sup>f</sup> J. Physiol., 1906, 34 : 84.

pletely or partly, of one particular metal, say calcium. If the proper ratios are not maintained in the blood, then:

(a) Is the excretion of calcium checked wholly or partially? During the progress of his research an article appeared by Goitein<sup>a</sup> which disposes of this question by showing that if a rabbit received less than 0.16 grams of calcium per kilo per day in its food, there was a steady loss of calcium from the body. Lehmann<sup>b</sup> and others have shown that in starvation the calcium excreted exceeds the amount of this substance present in the drinking water taken.

(b) Are the other salts of the body reduced *pari passu* by increased excretion? This would entail a considerable fall in the total molecular concentration of the blood, and as the living cells of the body and also the red corpuscles are extremely sensitive to osmotic changes this question may also be answered in the negative.

(c) Is the deficiency in the food made good by certain tissues of the body giving up a portion of their calcium to the blood and so keeping the proper inorganic balance in this fluid? That this would be the most probable contingency may be inferred from a number of facts. Forster,<sup>c</sup> who was the first to make observations on the effect of insufficient calcium in the food, found that the muscles lost 56 per cent of their calcium content, while the bones also showed a considerable diminution. Voit<sup>d</sup> found that on a calcium-poor diet the bones were more brittle, the skeleton showed a smaller percentage of dry weight than in the normal animal, and that the quantity of calcium in all organs of the body was more or less diminished.

In the experiments in which rabbits were fed on oatmeal and maize meal, a diet which admittedly leads to calcium starvation, the ratio of the calcium of the blood to the total ash of the blood remained the same as that found in the normal animal. That is to say, the blood underwent no loss of calcium relative to the other salts in the time allotted to the experiment—a result which one might anticipate from the immense importance of the salt ratios of the blood. The ratio of calcium to the total mineral matter in the bones was, however, inconstant, and showed fairly wide fluctuations even in the normal animal. The bones can, without doubt, act as store-houses of calcium and possibly of magnesium. That they lose calcium when the animal is placed on a calcium-poor diet has been proved conclusively. Voit's results, however, tend to show that the bones can lose calcium relatively to the other salts, that is, by a selective autolysis. The experiments on his own body metabolism show that calcium can be readily stored during nitrogen retention.

<sup>a</sup> Arch. gesam. Physiol., 1906, 115:118.

<sup>c</sup> Maly's Jahres-Ber., 1873, 3:251.

<sup>b</sup> Abs., Maly's Jahres-Ber., 1894, 23:497.

<sup>d</sup> Zts. Biol., 1880, 16:55.

More interesting, however, are the experiments involving rectal feeding, calcium being stored despite a continuous drainage of nitrogen from the body. In the latter case, as the protein absorbed from the food was insufficient, the muscles and glands must have diminished in bulk, and yet calcium was retained. This fact rather points to the bones as the place where calcium is stored. In the experiments on himself, and in those with rectal feeding, with a fixed diet the urinary calcium varied but slightly, and the variations, such as there were, ran parallel with the total amounts of urine excreted. This result is not remarkable if it is assumed that the kidney, in order to lighten its work against osmotic pressure, allows a fraction of each of the salts of the blood to escape into the urine. The greater the volume of the urine, therefore, the greater the amount of salts eliminated.

The following theories have been published by Albu and Neuberger<sup>a</sup> concerning the cause of rickets:

1. An insufficient amount of calcium in the food.
2. An inadequate absorption of the calcium salts of the food.
3. A disturbance of calcium retention in the bone-building tissues.
4. A disturbance of calcium absorption in bones themselves (Pfaundler).<sup>b</sup>
5. A connection between rickets and blood pressure based on the theory (Stöltzner)<sup>c</sup> that calcium metabolism is regulated by a secretion of the kidney.

Similar theories as to the cause of osteomalacia were enumerated by the same author as follows:

1. A lack of calcium in the food.
2. A lack of calcium absorption from the food.
3. A decreased alkalinity of blood, following an excess of free acids in the blood which dissolve the calcium salts of the bone.
4. A perversion of metabolism, (Fehling),<sup>d</sup> resulting from a diminished activity of the ovaries, which in time affects the calcium balance.
5. Hoenicke<sup>e</sup> classes osteomalacia as a metabolism disease, the phosphorus metabolism being also affected.

In pathological cases the results and opinions are many and diverse in regard to calcium elimination. For example, Beneke<sup>f</sup> found increased calcium elimination in fever, while Senator<sup>g</sup> obtained opposite results. In characteristic bone diseases, osteomalacia and rickets, the same state of affairs is found.

Calcium and magnesium occur in the urine for the most part as phosphates. The quantity of earthy phosphates eliminated daily is

<sup>a</sup> Mineralstoffwechsel, Berlin, 1906.

<sup>b</sup> Münch. med. Wochenschr., 1903, 50 : 1577.

<sup>c</sup> Jahrbuch f. Kinderheilkunde, 1900, 51 : 73.

<sup>d</sup> Arch. Gynaek., 1890-1, 39 : 171; 1894-5, 48 : 472.

<sup>e</sup> Berlin. klin. Wochenschr., 1904, 41 : 1154.

<sup>f</sup> Grundlinien der Pathologie des Stoffwechsels, Berlin, 1874.

<sup>g</sup> Centrbl. med. Wissensch., 1877, 15 : 357.

somewhat more than 1 gram, and of this amount two-thirds is magnesium and one-third calcium phosphate. In acid urines the simple as well as the double acid earthy phosphates are found, and the solubility of the former (among which the calcium salt,  $\text{CaHPO}_4$ , is especially insoluble) is particularly augmented by the presence of double acid alkali phosphates and sodium chlorid in the urine (Ott).<sup>a</sup> The quantity of alkaline earths in the urine depends upon the composition of the food.

### MAGNESIUM COMPOUNDS.

The relative ratio of magnesium to calcium as eliminated by the body is 1 : 8 or 1 : 9, and consists largely of magnesium phosphate,  $\text{Mg}_3(\text{PO}_4)_2$ . The amount of magnesium required by the body per day is 0.6 gram. As in the case of iron, though magnesium is necessary to health, but little magnesium is found in the child's food, namely, milk. The need of magnesium in the system has been studied by Bunge.<sup>b</sup> The magnesium balances have been studied by Blauberg,<sup>c</sup> Cronheim and Müller,<sup>d</sup> Bertram,<sup>e</sup> and Renvall,<sup>f</sup> but are not considered as important as the calcium. Moreover, little study has been given to the elimination of magnesium under pathological conditions.

The elimination of phosphoric acid, calcium, and magnesium depends principally on the character of the food and the relative proportion of animal and vegetable food digested.

### A FEEDING EXPERIMENT WITH RABBITS.

#### PLAN OF THE EXPERIMENT.

In these experiments four female rabbits were used, the diet containing as little phosphorus as possible. To two of the rabbits organic phosphorus in the form of crude phytin was fed, and to the other two an equivalent amount of phosphorus in the form of sodium phosphates was given.

It was intended to keep these four rabbits on their respective diets for three or four months, in order that they might become accustomed to the added phosphorus and, further, that it might be completely anabolized, and then to mate them and feed the young rabbits on the same kind of food and on phosphorus in the same respective combinations as that fed to the mother rabbits. When the young

<sup>a</sup> Zts. physiol. Chem., 1886, 10 : 1.

<sup>b</sup> Zts. Biol., 1874, 40 : 111, 295.

<sup>c</sup> Ibid., 1900, 40 : 1.

<sup>d</sup> Zts. diät. physik. Therapie, 1902-3, 6 : 25, 92.

<sup>e</sup> Abs., Chem. Centrbl., 1879, 10 : 526.

<sup>f</sup> Skand. Arch. Physiol., 1904, 16 : 94.

rabbits had lived for several weeks on these diets, it was planned to kill them and to examine their bodies in minutest detail for various combinations of nitrogen and phosphorus. The same procedure was to be carried out in the case of the four female rabbits, and in addition, normal rabbits were to be examined as controls. Unfortunately, it proved impossible to obtain young rabbits under these abnormal conditions, that is, living in closely confined quarters (cages) and fed on an artificial diet.

The work was begun early in November, 1907, and concluded the middle of March, 1908. Complete nitrogen<sup>a</sup> and phosphorus balances were determined during a period of nearly five months. Moreover, the inorganic phosphorus was estimated in the urine by the uranium acetate method throughout the entire time. In addition, during the last four weeks, calcium, magnesium, and ether-alcohol soluble phosphorus (lecithin) balances were included to make the study of the phosphorus metabolism more complete.

At the end of the period the rabbits were chloroformed, and the bones, teeth, blood, livers, nerves (including the spinal cord) and brains were analyzed for nitrogen, total phosphoric acid, lecithin-phosphoric acid, calcium, magnesium, water, ash, and ether extract. Two normal female rabbits were chloroformed and the same procedure followed as in the case of the rabbits artificially fed. In all cases post-mortem examinations were made and slides of the various tissues were prepared and histological changes noted.

#### PREPARATION OF FOOD.

The food consisted of carrots, gluten, a mixture of starch and sugar, olive oil, and salt solution. The above constituents seemed to furnish a well-rounded ration, supplying sufficient protein, fat, and carbohydrate for the needs of the body. The rabbits to which the inorganic phosphorus salts were fed received daily 5 cc of a standard salt mixture consisting of 450 grams of sugar, 4 grams of calcium chlorid, 15 grams of sodium chlorid, 30 grams of potassium chlorid, and 1 gram of magnesium sulphate, made up to a volume of 2,000 cc and containing 0.0492 gram of phosphoric acid, in the form of disodium hydrogen phosphate and sodium dihydrogen phosphate, per cubic centimeter.

The rabbits to which the organic phosphorus was fed received daily 5 cc of a salt mixture made so as to supply an equivalent amount of the above mineral salts, allowance being made for the presence of calcium, magnesium, potassium, and phosphorus in the phytin. In this way an equal amount of calcium, magnesium, potassium, and

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<sup>a</sup> All the nitrogen work was done by the nitrogen laboratory, Mr. T. C. Trescot in charge.

sodium was given to all four rabbits, and the total amount of phosphoric acid fed was practically equalized.

Gluten was selected as a food high in nitrogen but containing little phosphoric acid. The usual method of washing out the starch from coarse flour was employed. The moist gluten was spread out on sheets of tin and dried on the steam bath. After several days of this treatment the samples were sufficiently dried for grinding, and contained from 12 to 13 per cent of nitrogen.

The organic phosphorus was supplied in the form of phytin, a calcium-magnesium-potassium compound of anhydro-oxy-methylenedi-phosphoric acid which was first isolated by Posternak.<sup>a</sup> This was prepared by extracting wheat bran with 0.2 per cent hydrochloric acid, allowing the starch to settle, decanting off the clear liquid, and to this adding a large volume of 95 per cent alcohol. A heavy flocculent precipitate formed. This was allowed to settle and after the clear liquid had been decanted off, the remainder was filtered. The precipitate was then dried at room temperature by blowing air over it by means of an electric fan. In this air-dried condition the phytin contained from 22 to 30 per cent of phosphorus ( $P_2O_5$ ) in organic form. The uranium acetate titration method showed that no inorganic phosphorus was present.

The nature of phytin has been investigated by Patten and Hart,<sup>b</sup> who gave to it the following composition: Calcium, 1.13; magnesium, 5.80; and phosphorus, 16.3 per cent.

Phytin on heating with mineral acids is decomposed into inositol and phosphoric acid. The investigators just quoted claim there is no decomposition of phytin by enzymes and the same conclusion was reached by Mendel and Underhill,<sup>c</sup> who also studied this question. It is claimed that the proteolytic enzymes of the alimentary tract do not alter phytin, but that the alteration is brought about by the intestinal epithelium. The free acid phytin corresponds to the formula  $C_2H_8P_2O_9$ . The alkali salts are freely soluble in water and the calcium and copper salts are slightly soluble in water, while the barium and strontium salts are but sparingly soluble in water. Phytin has thus far been found in peas, beans, pumpkin seeds, and red and yellow lupines. The carbohydrates of the food were supplied by feeding a mixture consisting of equal portions of cane sugar and cornstarch. The fat used was olive oil.

The food was prepared in the following manner: The carrots were first chopped into small pieces and a portion was mixed with part of the gluten-starch-sugar mixture. To this was added the phytin and 5 cc of the phosphoric-acid-free salt solution in the case of the rabbits

<sup>a</sup> Rev. gen. bot., 1900, 12 : 5.

<sup>b</sup> Amer. Chem. J., 1904, 31 : 564.

<sup>c</sup> Amer. J. Physiol., 1906, 17 : 75.



fed organic phosphorus. This was made into a thick paste and placed on a small tray in one corner of the cage. The same procedure was followed in the case of the rabbits fed inorganic phosphorus, except that instead of phytin the salt solution containing the inorganic phosphorus was mixed with the food. Thus the rabbits were compelled to eat the food containing the phosphorus before the remainder of the food was given to them. The rest of the carrots, sugar and starch mixture, and 2 cc of olive oil were made into a thick paste and given to the rabbits during the afternoon.

#### METHODS OF ANALYSIS.

Each rabbit was confined in a suitable wire cage, which allowed the feces and urine to be easily separated.

After establishing a nitrogen equilibrium, the experiments with the rabbits were commenced. The feces were collected at frequent intervals during the day, owing to the fact that the rabbits persistently ate them. The urine and the food residues were collected daily. All of these samples were composited and analyzed; the nitrogen in the food, feces, and urine being determined according to the Gunning<sup>a</sup> method and the phosphoric acid in the food, feces, and urine by Neumann's<sup>b</sup> method. The phosphoric acid in the urine was determined also by the uranium acetate volumetric method. In this way a check on the amount of phosphoric acid in the urine was obtained, and further, this double determination served as an indication of the presence of organic phosphorus.

The methods employed for water and ash, and for calcium and magnesium<sup>c</sup> were those of the Association of Official Agricultural Chemists. From 2 to 3 grams of the foods or feces were ashed; and in the case of the urine, 200 cc were evaporated to dryness in a platinum dish and ashed. The ether-alcohol soluble phosphorus (lecithin) was determined in the following manner:

Transfer one or more grams of the finely ground substance to a 300-cc Erlenmeyer flask; add 30 cc of absolute ether and extract the whole over night. Filter the ether extract through a hardened filter paper into an ordinary Jena flat-bottomed flask of 500-cc capacity and return any particles of the residue found on the filter paper to the Erlenmeyer flask. Then add to the ether extract residue 60 cc of absolute alcohol and boil the solution for three hours, using a reflux condenser. Filter this alcohol extract while hot into the Jena flask containing the ether extract and wash the residue twice with two separate portions of 25 cc each of hot alcohol, adding the washings to the extract. In the combined ether-alcohol filtrate determine the phosphoric acid by the Neumann<sup>b</sup> method.

<sup>a</sup> U. S. Dept. Agr., Bureau of Chemistry, Bul. 107, Rev., p. 7.

<sup>b</sup> Zts. physiol. Chem., 1902, 37 : 115.

<sup>c</sup> U. S. Dept. Agr., Bureau of Chemistry, Bul. 107, Rev., p. 15, 16.

## PRELIMINARY FEEDING PERIOD.

The results recorded in Table I are especially valuable in view of the fact that they cover a period of practically one hundred days. During this time an attempt was made to mate the rabbits, each one being removed from her cage every four days at 5 o'clock and placed in a large box with a male rabbit and allowed to remain there until the next morning at 9 o'clock. This was repeated fifteen or twenty times in each case. Consequently some loss of feces and urine must have resulted, which loss in the course of an experiment extending over one hundred days would be practically uniform in the case of each pair of rabbits. Such a loss would naturally tend to give somewhat larger apparent nitrogen and phosphoric acid balances. The rabbits had always eaten the food provided for them before being removed from their cages.

Throughout this work the rabbits fed organic phosphorus are given the numbers 1 and 2, and those fed inorganic phosphorus the numbers 3 and 4.

The weight of the rabbits remained constant in the case of Nos. 1 and 4, while in the case of No. 2 a slight average loss of weight resulted and No. 3 showed a gain in weight.

TABLE I.—*Nitrogen and phosphorus metabolism—Preliminary period.*

No. 1.—RABBIT FED ORGANIC PHOSPHORUS.

Date.	Average weight of rabbit.	Nitrogen (N).				Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ).				In urine.		Absorbed material retained.		
		Total ingested.		Total excreted.		Daily balance.	Total ingested. <sup>a</sup>		Excreted.		Nitrogen.	Phosphoric acid.	Nitrogen.	Phosphoric acid.
		In urine.	In feces.	In urine.	In feces.		Daily balance.	Daily balance.						
1907-8.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	P. ct.	P. ct.	P. ct.	P. ct.	
November 17-23.....	1,647	3.34	4.22	0.40	-0.18	0.66	0.95	0.28	-0.08	.....	.....	.....	.....	
November 24-30.....	1,596	5.35	4.65	.92	-.03	1.81	.13	.90	.11	.....	.....	.....	.....	
December 1-7.....	1,596	8.22	4.91	.83	.35	2.37	.77	.76	.11	.....	.....	.....	.....	
December 8-14.....	1,654	9.33	5.22	.39	.53	3.13	.50	.49	.30	.....	.....	.....	.....	
Average.....	1,623	6.56	4.75	.64	.17	1.99	.59	.61	.11	72.40	29.65	.....	.....	
December 15-21.....	1,656	10.10	4.78	.60	.68	3.29	.68	.69	.27	.....	.....	.....	.....	
December 22-28.....	1,663	10.10	6.33	.60	.45	3.29	1.00	.69	.22	.....	.....	.....	.....	
December 29-January 4.....	1,677	3.54	5.26	.85	-.36	1.74	1.12	.77	-.02	.....	.....	.....	.....	
January 5-11.....	1,623	7.44	5.12	.54	.25	2.79	.81	.44	.21	.....	.....	.....	.....	
January 12-18.....	1,616	7.03	4.58	1.12	1.17	2.67	.63	1.95	.14	.....	.....	.....	.....	
Average.....	1,647	7.64	5.21	.75	.24	2.76	.85	.91	.16	68.19	30.79	.....	.....	
January 19-25.....	1,597	9.56	6.40	1.08	.29	3.27	.77	1.01	.21	.....	.....	.....	.....	
January 26-February 1.....	1,587	10.06	6.06	1.32	.38	3.24	1.04	1.05	.16	.....	.....	.....	.....	
February 2-8.....	1,588	10.19	7.34	1.02	.26	3.27	1.11	1.02	.16	.....	.....	.....	.....	
February 9-15.....	1,567	10.60	8.11	1.33	.16	3.23	1.80	1.16	.03	.....	.....	.....	.....	
Average.....	1,585	10.10	6.98	1.19	.27	3.25	1.18	1.06	.14	69.11	34.71	.....	.....	
Average for period.	1,618	8.08	5.65	.86	.23	2.67	.87	.86	.14	69.90	31.72	22	53	

<sup>a</sup> 1.42 grams of organic phosphorus per period were intimately mixed with the food.

TABLE I.—Nitrogen and phosphorus metabolism—Preliminary period—Continued.

## No. 2.—RABBIT FED ORGANIC PHOSPHORUS.

Date.	Average weight of rabbit.	Nitrogen (N).				Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ).				In urine.		Absorbed material retained.	
		Total ingested.	Total excreted.		Daily balance.	Total ingested. <sup>a</sup>	Excreted.		Daily balance.	Nitrogen.	Phosphoric acid.	Nitrogen.	Phosphoric acid.
			In urine.	In feces.			In urine.	In feces.					
<i>1907-8.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
November 17-23.....	1,816	4.16	4.86	0.51	0.17	1.35	0.82	0.45	0.61	.....	.....	.....	.....
November 24-30.....	1,728	4.32	6.09	.56	-.33	1.98	.39	.41	.16	.....	.....	.....	.....
December 1-7.....	1,684	9.97	4.96	.76	.60	2.88	.65	.72	.21	.....	.....	.....	.....
December 8-14.....	1,618	9.95	6.78	1.75	.20	3.26	.66	1.35	.17	.....	.....	.....	.....
Average.....	1,712	7.10	5.67	.90	.16	2.37	.63	.86	.14	79.86	26.58	.....	.....
December 15-21.....	1,542	11.76	7.82	2.26	.23	3.37	.93	1.88	.05	.....	.....	.....	.....
December 22-28.....	1,480	11.76	7.84	2.26	.23	3.37	.88	1.88	.08	.....	.....	.....	.....
December 29-January 4.....	1,505	10.67	5.85	1.19	.51	3.36	.31	.95	.29	.....	.....	.....	.....
January 5-11.....	1,567	10.67	5.40	1.58	.52	3.36	.62	1.60	.16	.....	.....	.....	.....
January 12-18.....	1,646	10.67	5.61	.57	.64	3.36	.41	.54	.34	.....	.....	.....	.....
Average.....	1,548	11.11	6.50	1.57	.43	3.36	.63	1.37	.18	58.51	18.75	.....	.....
January 19-25.....	1,658	11.12	5.55	1.29	.60	3.33	.83	1.24	.18	.....	.....	.....	.....
January 26-February 1.....	1,698	11.71	6.48	1.51	.44	3.31	1.18	1.01	.15	.....	.....	.....	.....
February 2-8.....	1,697	9.11	7.75	2.84	.21	2.63	1.76	1.26	.05	.....	.....	.....	.....
February 9-15.....	1,647	8.85	6.65	.83	.19	2.90	1.20	.65	.14	.....	.....	.....	.....
Average.....	1,675	10.20	6.61	1.62	.36	3.04	1.24	1.04	.11	64.80	40.79	.....	.....
Average for period.	1,645	9.47	6.26	1.39	.32	2.92	.83	1.09	.14	67.72	28.71	24	52

No. 3.—RABBIT FED INORGANIC PHOSPHORUS.<sup>b</sup>

November 17-23.....	1,474	7.53	6.48	1.20	-0.02	2.83	0.58	1.02	0.16	.....	.....	.....	.....
November 24-30.....	1,481	9.10	7.46	2.15	-.07	3.27	.96	1.38	.13	.....	.....	.....	.....
December 1-7.....	1,447	10.40	8.89	1.12	.05	3.32	.98	1.38	.13	.....	.....	.....	.....
December 8-14.....	1,436	10.05	7.20	1.15	.24	3.59	.99	1.15	.20	.....	.....	.....	.....
Average.....	1,460	9.27	7.51	1.41	.05	3.25	.88	1.24	.16	81.01	27.08	.....	.....
December 15-21.....	1,401	11.70	5.48	.65	.79	3.67	.84	.25	.36	.....	.....	.....	.....
December 22-28.....	1,484	11.70	6.14	.65	.70	3.67	1.54	.25	.26	.....	.....	.....	.....
December 29-January 4.....	1,526	10.62	4.73	1.66	.60	3.66	.63	.99	.30	.....	.....	.....	.....
January 5-11.....	1,598	10.62	6.50	1.50	.37	3.66	1.08	1.27	.18	.....	.....	.....	.....
January 12-18.....	1,652	10.62	4.80	.88	.69	3.66	.82	.88	.27	.....	.....	.....	.....
Average.....	1,532	11.05	5.55	1.07	.63	3.66	.98	.73	.27	50.23	26.78	.....	.....
January 19-25.....	1,729	11.06	5.82	1.18	.57	3.64	1.06	1.17	.20	.....	.....	.....	.....
January 26-February 1.....	1,766	11.65	5.72	1.62	.61	3.61	1.16	1.58	.12	.....	.....	.....	.....
February 2-8.....	1,776	11.65	6.51	2.54	.37	3.61	1.28	1.97	.04	.....	.....	.....	.....
February 9-15.....	1,778	12.08	7.65	2.54	.26	3.65	.85	1.98	.11	.....	.....	.....	.....
Average.....	1,762	11.61	6.43	1.97	.45	3.63	1.09	1.67	.12	55.38	30.02	.....	.....
Average for period.	1,585	10.64	6.50	1.48	.38	3.51	.98	1.37	.18	62.21	27.96	29	54

<sup>a</sup> 1.42 grams of organic phosphorus per period were intimately mixed with the food.<sup>b</sup> 1.72 grams of inorganic phosphorus per period were intimately mixed with the food.

TABLE I.—Nitrogen and phosphorus metabolism—Preliminary period—Continued.

No. 4.—RABBIT FED INORGANIC PHOSPHORUS.

Date.	Average weight of rabbit.	Nitrogen (N).				Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ).				In urine.		Absorbed material retained.	
		Total ingested.	Total excreted.		Daily balance.	Total ingested. <sup>a</sup>	Excreted.		Daily balance.	Nitrogen.	Phosphoric acid.	Nitrogen.	Phosphoric acid.
			In urine.	In feces.			In urine.	In feces.					
1907-8.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	P. ct.	P. ct.	P. ct.	P. ct.	
November 17-23.....	1,950	8.90	8.89	0.76	-0.10	3.10	1.54	0.49	0.15	.....	.....	.....	.....
November 24-30.....	1,960	9.14	7.91	1.13	.01	3.31	1.94	.94	.20	.....	.....	.....	.....
December 1-7.....	1,960	10.40	4.81	.42	.73	3.32	1.19	.36	.25	.....	.....	.....	.....
December 8-14.....	1,985	10.05	3.71	.68	.80	3.59	.36	.51	.38	.....	.....	.....	.....
Average.....	1,964	9.62	6.33	.75	.36	3.33	1.34	.58	.25	65.80	40.24	.....	.....
December 15-21.....	1,965	11.70	5.16	1.06	.78	3.67	.74	.90	.28	.....	.....	.....	.....
December 22-28.....	2,002	11.70	8.63	1.06	.32	3.67	1.42	.90	.19	.....	.....	.....	.....
December 29-January 4.....	1,953	10.62	8.89	1.28	.06	3.66	1.44	1.42	.11	.....	.....	.....	.....
January 5-11.....	1,952	10.62	6.44	.26	.55	3.66	1.02	.42	.31	.....	.....	.....	.....
January 12-18.....	2,022	10.62	5.21	.46	.70	3.66	1.04	.57	.29	.....	.....	.....	.....
Average.....	1,979	11.05	6.86	.82	.48	3.66	1.13	.84	.24	62.08	30.88	.....	.....
January 19-25.....	2,006	7.78	5.94	1.09	.10	3.20	1.55	1.09	.04	.....	.....	.....	.....
January 26-February 1.....	2,017	11.65	7.43	.72	.50	3.61	1.29	.77	.22	.....	.....	.....	.....
February 2-8.....	2,000	7.06	5.44	.46	.16	2.28	1.48	.54	.03	.....	.....	.....	.....
February 9-15.....	1,975	8.07	6.59	2.38	-.12	1.45	1.03	2.36	-.13	.....	.....	.....	.....
Average.....	2,000	8.64	6.35	1.16	.16	2.64	1.34	1.19	.04	73.50	50.76	.....	.....
Average for period.	1,981	9.77	6.51	.91	.33	3.21	1.27	.87	.18	67.12	40.63	26	42

<sup>a</sup> 1.72 grams of inorganic phosphorus per period were intimately mixed with the food.

## NITROGEN BALANCES.

The rabbit which gained in weight, No. 3, received a larger amount of nitrogen than the others. In fact, the amount of nitrogen ingested in all cases varied somewhat, but the total during a period of seven days was from 5 to 6.6 grams of nitrogen per 1,000 grams of body weight, No. 3 receiving the largest relative amount. The average figures show that relatively more nitrogen was excreted in the urine in the cases of rabbits No. 1 and No. 2 (those fed organic phosphorus), than in the cases of No. 3 and No. 4 (fed inorganic phosphorus). The amount of nitrogen eliminated in the feces varied with the individual rabbit. No. 1 eliminated 10.6 per cent and No. 2, 14.6 per cent. No. 3 eliminated 13.8 per cent and No. 4 only 9.3 per cent in this manner. Nos. 1 and 2 retained a smaller proportion of the metabolized nitrogen than did Nos. 3 and 4, the figures being respectively 22, 24, 29, and 26 per cent. This means that the rabbits fed inorganic phosphorus retained a larger proportion of the absorbed nitrogen than did those fed organic phosphorus; and it appears that those fed organic phosphorus excreted in the urine a larger proportion of the ingested nitrogen, but did not utilize this nitrogen so well as did the rabbits fed inorganic phosphorus.

## PHOSPHORUS BALANCES.

In all cases, excepting rabbit No. 3, the average amount of phosphoric acid ingested during seven days per 1,000 grams of body weight varied from 1.6 to 1.7 grams; in No. 3 the amount was 2.2 grams. More phosphorus was eliminated by rabbit No. 4 through the kidneys than in any other case. Both of the rabbits fed organic phosphorus and No. 3 fed inorganic phosphorus retained about the same amounts of the absorbed phosphorus (averaging 53 per cent), while the figure for No. 4 is much lower, only 42 per cent. From the average figures, it appears that the rabbits fed organic phosphorus eliminated a smaller percentage of the ingested phosphoric acid in the urine than those fed on inorganic phosphorus. It must be remembered that the amount of phosphoric acid eliminated by the kidneys does not necessarily represent the amount metabolized, for inorganic phosphorus ingested might and undoubtedly does pass through the kidneys without undergoing any change. Of the total phosphoric acid ingested, 32 per cent was found in the feces of No. 1 and 31 per cent in the feces of No. 4, while No. 2 and No. 3 eliminated in this manner 37 and 39 per cent, respectively. Although the rabbits fed inorganic phosphorus excreted a larger amount of phosphorus in the urine than did the others, they retained on the average less of the absorbed phosphorus.

The ratio of nitrogen to phosphoric acid in the food is but slightly above 3:1. This shows a much larger proportion of phosphoric acid than is usually fed in a normal diet. The ratio of nitrogen to phosphoric acid in the urine varies from 5:1 to 7.5:1, being higher in the case of the rabbits fed organic phosphorus, owing to the relatively larger elimination of phosphoric acid in the urine of those fed inorganic phosphorus. This ratio in the feces is rather constant, averaging about 1.1:1 in all cases. The exact ratios are given in Table II.

TABLE II.—*Ratio of nitrogen to phosphoric acid in food and excreta—Preliminary period.*

Rabbit.	Food.	Urine.	Feces.
No. 1.....	3.03:1	6.50:1	1.00:1
No. 2.....	3.24:1	7.54:1	1.27:1
No. 3.....	3.03:1	6.63:1	1.08:1
No. 4.....	3.04:1	5.12:1	0.91:1

## PRINCIPAL FEEDING PERIOD.

The principal feeding experiment extended over a period of four weeks, and during this time complete nitrogen, phosphoric acid, calcium, and magnesium balances were determined, as well as the ether-alcohol soluble phosphorus balance. In addition the inorganic phosphorus in the urine was determined by the uranium-acetate titration method. Other salts, as well as the calcium and magnesium salts,

are important for the welfare of the organism, but in phosphorus-feeding experiments the two named stand out most prominently. The second rabbit fed organic phosphorus was in poor condition during the test and died of pneumonia at the end of the second week; consequently the results in this case must not be given the same weight as in the others. Throughout this period all of the rabbits remained practically constant in weight. Nos. 3 and 4 received more nitrogen and phosphoric acid than did Nos. 1 and 2 on an average, but Nos. 1 and 4 received practically the same amounts. The nitrogen and phosphorus balances were positive, except in the case of rabbit No. 2, which died, and naturally would show a negative set of balances.

## NITROGEN BALANCES.

The analytical data obtained during the experiment are recorded in Table III.

TABLE III.—Nitrogen and phosphoric acid balances—Principal period.

## RABBITS FED ORGANIC PHOSPHORUS.

Date.	Rabbit.	Total ingested.		Total excreted.				Daily balance.		In urine.		Absorbed material retained.		
		Average weight.	Nitrogen.	Phosphoric acid.	Nitrogen.		Phosphoric acid.		Nitrogen.	Phosphoric acid.	Nitrogen.	Phosphoric acid.	Nitrogen.	Phosphoric acid.
					In feces.	In urine.	In feces.	In urine.						
1908.		Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	P. ct.	P. ct.	P. ct.	P. ct.	
February 17-23.....	1	1,565	9.891	2.824	0.751	6.471	0.602	0.887	0.381	0.191	.....	.....	.....	.....
February 24-.....														
March 1.....	1	1,551	10.176	3.078	2.449	6.680	1.569	.970	.149	.077	.....	.....	.....	.....
March 2-8.....	1	1,551	10.745	3.391	1.482	7.560	.998	1.146	.243	.178	.....	.....	.....	.....
March 9-15.....	1	1,533	10.589	3.483	2.310	6.470	1.461	.489	.253	.218	.....	.....	.....	.....
Average.....	1	1,550	10.3498	3.194	1.748	6.795	1.157	.876	.256	.166	65.65	27.41	21.0	57.1
February 17-23.....	2	1,660	7.583	2.626	1.572	7.087	.900	1.307	-.154	.060	.....	.....	.....	.....
February 24-.....														
March 1.....	2	1,623	8.519	2.451	3.284	7.480	1.738	1.230	-.374	-.085	.....	.....	.....	.....
Average 2 weeks.....		1,642	8.051	2.539	2.828	7.284	1.319	1.269	-.264	-.013	90.47	49.97	.....	.....
General average.....		1,596	9.200	2.867	2.288	7.040	1.238	1.073	.....	.....	.....	.....	.....	.....

## RABBITS FED INORGANIC PHOSPHORUS.

February 17-23.....	3	1,765	12.398	3.688	2.378	6.959	1.766	1.345	0.437	0.082	.....	.....	.....	.....
February 24-.....														
March 1.....	3	1,791	12.414	3.688	2.434	7.429	1.479	1.495	.363	.102	.....	.....	.....	.....
March 2-8.....	3	1,814	12.149	3.944	2.632	7.882	1.328	1.295	.365	.140	.....	.....	.....	.....
March 9-15.....	3	1,814	11.962	3.944	1.133	7.882	.534	1.014	.421	.342	.....	.....	.....	.....
Average.....		1,796	12.231	3.788	2.144	7.308	1.328	1.295	.397	.166	59.75	34.17	27.5	47.3
February 17-23.....	4	1,986	9.157	3.206	.596	7.680	.592	1.800	.126	.116	.....	.....	.....	.....
February 24-.....														
March 1.....	4	2,028	12.401	3.688	.743	10.211	.440	1.927	.207	.189	.....	.....	.....	.....
March 2-8.....	4	2,011	12.249	3.933	1.198	7.520	.760	1.540	.505	.219	.....	.....	.....	.....
March 9-15.....	4	1,971	7.72	3.222	1.212	7.360	.728	1.806	-.114	.098	.....	.....	.....	.....
Average.....		1,994	10.395	3.487	.937	8.193	.630	1.768	.181	.156	78.82	50.71	13.4	38.1
General average.....		1,895	11.313	3.638	1.521	7.751	.979	1.532	.289	.161	69.29	42.44	20.5	42.7

<sup>a</sup> Including 1.38 grams of organic phosphorus added to the food per period.

<sup>b</sup> Including 1.42 grams of inorganic phosphorus added to the food per period.

The amount of nitrogen ingested per 1,000 grams of body weight for a seven-day period was as follows: No. 1; 6.7 grams; No. 3, 6.8 grams; No. 4, 5.2 grams. The amount of nitrogen absorbed per 1,000 grams of body weight per period of seven days was likewise very uniform, excepting for rabbit No. 2, being 5.6 grams for No. 1 and No. 3, 4.8 grams for No. 4, and 3.2 grams for No. 2. The amounts of nitrogen excreted in the urine and feces show considerable variation, the ratio of urine nitrogen to feces nitrogen being highest in No. 4, that is, 8.7:1, and lowest in No. 2, 2.6:1. No. 1 showed a ratio of 3.9:1, and No. 3 a ratio of 3.4:1.

In Table IV the ratios of nitrogen to phosphoric acid, calcium to magnesium, and phosphoric acid to calcium in food, feces, and urine are given. The relation of urine nitrogen to urine phosphorus is highest in the cases of the rabbits fed organic phosphorus. This is due to a larger excretion of phosphorus in the urine of rabbits Nos. 3 and 4, fed on inorganic phosphorus. This ratio in the feces is very regular, being 1.5:1 in three cases and for No. 2 increasing to 2.1:1. A higher ratio of calcium to magnesium is noted in the feces of the rabbits fed inorganic phosphorus. This ratio varies considerably in the urine of the individual rabbits.

TABLE IV.—*Ratios of calcium, magnesium, and phosphorus in food, feces, and urine—Principal period.*

RABBITS FED ORGANIC PHOSPHORUS.				
Rabbit No.	In—	N:P <sub>2</sub> O <sub>5</sub> .	Ca:Mg.	P <sub>2</sub> O <sub>5</sub> :Ca.
1	Food.....	3.2	2.6	3.6
	Feces.....	1.5	2.7	2.0
	Urine.....	7.7	3.8	10.9
2	Food.....	3.2	3.0	3.2
	Feces.....	2.1	1.9	3.1
	Urine.....	5.7	1.0	14.7
RABBITS FED INORGANIC PHOSPHORUS.				
3	Food.....	3.2	2.9	4.2
	Feces.....	1.5	3.5	3.6
	Urine.....	5.5	2.2	34.0
4	Food.....	2.9	2.9	4.0
	Feces.....	1.5	3.7	1.8
	Urine.....	4.6	6.6	15.7

The figures show also that the phosphorus-calcium ratios of the food of rabbits Nos. 1 and 2 are lower than in the food of rabbits Nos. 3 and 4; this is due to a larger ingestion of phosphorus in the latter cases. The ratio of phosphoric acid to calcium in the feces varies with the individual case. The phosphoric acid to calcium ratios in the urine again show more phosphorus eliminated by rabbits Nos. 3 and 4 than by rabbits Nos. 1 and 2. The nitrogen and phosphoric acid eliminated in the urine are generally considered to represent the

amounts of the two substances metabolized by the system, but this does not hold in all cases. Rabbit No. 2, for example, which died, shows a much larger amount of katabolized nitrogen and phosphoric acid, as indicated by an increased elimination through the kidneys, but this does not indicate an increased metabolism of these two substances. The ratio of nitrogen to phosphoric acid excreted in the feces shows equal average figures for all the rabbits, excluding the figures for No. 2. If the figures for rabbit No. 2 are included, the average ratio is lower for the rabbits fed inorganic phosphorus. The bulk of the nitrogen (60 to 78 per cent) is eliminated by the kidneys, whereas, on the other hand, only 27 to 50 per cent of the phosphoric acid is thus eliminated. The percentage of absorbed nitrogen which was retained in the system is the same for rabbit No. 1 as for the average of Nos. 3 and 4, receiving inorganic phosphorus.

#### PHOSPHORUS BALANCES.

The study of phosphoric acid metabolism raises many questions, of which the following are especially important:

(1) How is the phosphoric acid, ingested in different forms, taken up by the body?

(2) How is the phosphoric acid changed in the body?

(3) In what manner is the phosphoric acid eliminated from the body?

Many investigators have attempted to answer some or all of these questions, but no definite answer has been obtained. The generally accepted idea is that the phosphoric acid ingested in different forms is taken up by the body partly in various forms of organic combination and partly, also, in the inorganic or phosphate form. Most of the organic phosphorus taken up by the body—that absorbed from the intestines—is changed to the inorganic or phosphate form, and all such phosphorus is eliminated in the urine as phosphates. This idea that organic phosphorus compounds are more valuable than inorganic combinations of phosphoric acid has been promulgated in the medical literature during the past few years. Nevertheless, many practicing physicians continue to prescribe the inorganic forms, not only of phosphorus, but of iron, calcium, magnesium, etc. Yellow phosphorus is given solely as an alterative.

As the extent of the elimination of phosphoric acid is largely dependent upon the character of the food and the absorption of the phosphates in the intestines, it is apparent that the relationship between the nitrogen and phosphoric acid in the urine can only be approximately constant with a certain uniform food. Thus, on feeding dogs with an exclusive meat diet, as observed by Voit,<sup>a</sup> when the

<sup>a</sup> Cited by Hammarsten, *A Textbook of Physiological Chemistry*, rev. ed., New York, 1908.



nitrogen and phosphoric acid of the food exactly reappeared in the urine and the feces, the relationship was 8.1:1. In these experiments with rabbits the nitrogen and phosphoric acid ratio in the urine varied from 4.6:1 to 7.7:1. In starvation Wellmann<sup>a</sup> has shown that this relationship is changed, namely, relatively more phosphoric acid is eliminated, which seems to indicate that besides flesh and related tissues, also another tissue rich in phosphorus is largely destroyed. The starvation experiments show that this is the bone tissue. Tigerstedt<sup>b</sup> claims that only 0.134 gram of phosphoric acid is eliminated in the feces of man daily. For some years it was claimed by many investigators that the elimination of phosphorus and nitrogen should run parallel, both substances being derived from protein, the usual ratio of nitrogen to phosphorus being 7.5:1. In these experiments there is a general tendency in the individual data toward parallelism between the nitrogen and phosphoric acid excretion in the urine, but this ratio is not maintained in the general average, as in the case of the rabbits fed inorganic phosphorus a much larger proportion of the phosphoric acid is absorbed and eliminated by the kidneys. Siven,<sup>c</sup> Ehrström,<sup>d</sup> and Meyer<sup>e</sup> have also shown no parallelism to exist. Phosphorus is used in the formation of the bones and other bodies where no nitrogen is present. Moreover, the ratio of nitrogen and phosphoric acid will sometimes run as low as 3:1.

The amount of phosphoric acid which was fed to all the rabbits was considerably higher than the amount present in their normal diet. In fact, the food itself contained practically a sufficient amount to supply the needs of the system. The result is that by adding an excess of phosphoric acid metabolic changes were induced in all cases to a greater or less extent. The amount of phosphoric acid fed per seven-day period per 1,000 grams of body weight varied from 1.6 grams in rabbit No. 2 to 2.1 grams in the cases of rabbits Nos. 1 and 3. The amount of absorbed phosphorus per 1,000 grams of body weight was practically equal, 1.3 grams, excepting in the case of rabbit No. 2, where the figures show 0.7 gram of phosphoric acid per 1,000 grams of body weight. The ratio of phosphoric acid in the urine to that in the feces shows that the individual element was the most important factor, rabbit No. 4 eliminating a far larger proportional amount by the kidneys than in the case of any other rabbit. The percentage of phosphoric acid eliminated by the kidneys was higher in the rabbits fed inorganic phosphoric acid than in rabbit No. 1, but this simply means that more of the inorganic phosphoric acid passed through the kidneys unaltered, for rabbit No. 1 retained a larger proportion of its absorbed phosphorus than did either No. 3 or No. 4.

<sup>a</sup> Arch. gesam. Physiol., 1908, 121:508.

<sup>d</sup> Ibid., 1903, 14:82.

<sup>b</sup> Skand. Arch. Physiol., 1904, 16:67.

<sup>e</sup> Zts. physiol. Chem., 1904-5, 43:1.

<sup>c</sup> Ibid., 1901, 11:308.

According to Ehrström,<sup>a</sup> who studied phosphorus elimination, from 50 to 88 per cent of the phosphoric acid is eliminated in the urine by the human organism, but the average amount given by different investigators varies from 70 to 80 per cent. With animal food almost all the phosphorus is eliminated in the urine, while with vegetable food a larger proportion of the phosphorus is found in the feces. The amounts of calcium and phosphorus present in the food stand in close relationship to one another. The rabbits eliminated from 27 to 50 per cent of the ingested phosphoric acid in the urine, a considerably lower percentage than in the case of carnivorous animals.

## ETHER-ALCOHOL-SOLUBLE PHOSPHORUS BALANCES.

The ether-alcohol soluble phosphorus balances were determined during the principal feeding period. The amount of phosphorus ingested in this form was practically the same in all cases. It is interesting to note the small portion of the 29 per cent of organic combined phosphorus in phytin, which is soluble in ether and alcohol. The figures show that 0.59 per cent of the phosphorus was present in phytin as ether-alcohol soluble phosphorus. This shows that the ether-alcohol extracted phosphorus may represent but a small proportion of the total organic combined phosphorus.

The presence of organic phosphorus in the urine has been discussed, but it is interesting to note that the average figures in Tables V and VI show a slightly larger amount of the so-called organic, or ether-alcohol soluble phosphorus in the urine in the case of the rabbits fed on inorganic phosphorus.

TABLE V.—*Ether-alcohol-soluble phosphoric acid balances—Principal period.*

## RABBITS FED ORGANIC PHOSPHORUS.

Date.	Number of rabbit.	Phosphoric acid ingested.	Excreted as phosphoric acid.			Phosphoric acid balance.	Total phosphoric acid in feces.	Ether-alcohol soluble phosphoric acid of feces in terms of total phosphoric acid.
			In urine.	In feces.	Total.			
1908.								
February 17-23.....	1	Gram. 0.2830	Gram. 0.0180	Gram. 0.0146	Gram. 0.0596	Gram. 0.2380	Grams. 0.6020	Per cent. 2.4
February 24-March 1.....	1	.2923	.0050	.0795	.0845	.2075	1.5680	5.1
March 2-8.....	1	.3081	.0036	.0992	.1028	.2053	.9976	9.9
March 9-15.....	1	.3080	.0000	.1188	.1188	.1893	1.4608	8.1
Average.....		.2994	.0067	.0848	.0914	.2100	1.1571	6.4
February 17-23.....	2	.2413	.0162	.0873	.1035	.1378	.9000	9.3
February 24-March 1.....	2	.2374	.0110	.1925	.2035	.0339	1.7380	20.8
Average.....		.2394	.0136	.1399	.1535	.0859	1.3190	15.1
General average.....		.2694	.0102	.1089	.1225	.1479	1.2381	10.8

<sup>a</sup> Skan. Arch. Physiol; 1903, 14: 82.

TABLE V.—Ether-alcohol-soluble phosphoric acid balances—Principal period—Continued.

RABBITS FED INORGANIC PHOSPHORUS.

Date.	Number of rabbit.	Phosphoric acid ingested.	Excreted as phosphoric acid.			Phosphoric acid balance.	Total phosphoric acid in feces.	Ether-alcohol soluble phosphoric acid of feces in terms of total phosphoric acid.
			In urine.	In feces.	Total.			
<i>1908.</i>								
February 17-23.....	3	Gram. 0.2797	Gram. 0.0133	Gram. 0.0175	Gram. 0.0308	Gram. 0.2489	Grams. 1.7664	Per cent. 1.0
February 24-March 1.....	3	.2797	.0138	.0232	.0370	.2427	1.4792	1.6
March 2-8.....	3	.2797	.0080	.0248	.0328	.2469	1.5308	1.6
March 9-15.....	3	.2797	.0000	.0090	.0090	.2707	.5340	1.7
Average.....		.2797	.0088	.0187	.0274	.2523	1.3276	1.5
February 17-23.....	4	.2629	.0081	.0386	.0467	.2162	.5920	6.5
February 24-March 1.....	4	.2797	.0336	.0166	.0502	.2295	.4400	3.8
March 2-8.....	4	.2797	.0180	.0930	.1110	.1687	.7600	12.2
March 9-15.....	4	.2797	.0000	.0322	.0322	.2477	.7280	4.4
Average.....		.2755	.0149	.0451	.0600	.2130	.6300	6.7
General average.....		.2776	.0119	.0319	.0437	.2327	.9788	4.1

The figures also show that in the case of the rabbits fed organic phosphorus a considerable amount of ether-alcohol soluble phosphorus is excreted in the feces. The analyses of the feces of the rabbits fed inorganic phosphorus show that there is some ether-alcohol soluble phosphorus always present. This amount must come from the ether-alcohol soluble phosphorus of the food, or from the secretions of the intestinal juices. There is no doubt that the feeding of phytin, which contains 0.59 per cent of phosphorus in this form, greatly increases the amount of ether-alcohol soluble phosphorus in the feces. The insoluble calcium phosphate formed in the gastro-intestinal tract of the rabbits fed inorganic phosphorus may tend to give a higher ratio of ether-alcohol soluble phosphorus in the feces of the rabbits fed with phytin. Further, the results indicate that of the total phosphorus eliminated in the feces the percentage of ether-alcohol soluble phosphorus is much larger in the case of the rabbits fed on organic phosphorus. This may indicate that when phytin is fed in large amounts the gastro-intestinal tract is not able to split and absorb it all. Consequently the percentage of ether-alcohol soluble phosphorus in the feces is increased. The question of the presence of organic phosphorus in the urine is still unsettled. In the principal feeding period the phosphorus in the urine was determined by the uranium acetate titration method, and, further, the lecithin phosphorus, ether-alcohol method, was applied to the urine after evaporating 100 cc to dryness. The figures which are given in Table VI show that the urine results for phosphorus obtained

by the different methods are practically the same whether organic or inorganic phosphorus is fed.

TABLE VI.—*Various forms of phosphorus in urine—Principal period.*

Date.	Number of rabbit.	Phosphoric acid per 100 cc of urine.				Difference between two figures for organic phosphorus.
		Total (by Neumann method).	Inorganic (by uranium acetate method).	Organic (by difference).	Organic (by ether-alcohol extraction).	
<i>1908.</i>						
February 17-23.....	1	Gram. 0.0986	Gram. 0.1009	Gram. —0.0023	Gram. 0.0020	Gram. 0.0043
February 24-March 1.....	1	.....	.1153	.....	.0905	.....
March 2-8.....	1	.....	.1080	.....	.0903	.....
March 9-15.....	1	.....	.0670	.....	.0000	.....
February 17-23.....	2	.1376	.1308	.0068	.0017	.0051
February 24-March 1.....	2	.1280	.1184	.0046	.0011	.0035
February 17-23.....	3	.1416	.1277	.0139	.0014	.0125
February 24-March 1.....	3	.1300	.1287	.0013	.0012	.0001
March 2-8.....	3	.1325	.1290	.0035	.0008	.0027
February 17-23.....	4	.2250	.2039	.0211	.0009	.0202
February 24-March 1.....	4	.1835	.1740	.0095	.0332	.0063
March 2-8.....	4	.1540	.1340	.0200	.0018	.0182
March 9-15.....	4	.1570	.1290	.0280	.....	.....

An ether-alcohol extraction of an inorganic phosphate solution (100 cc of a microcosmic salt solution) was made containing 0.2 gram of phosphoric acid and this gave 0.0054 gram of phosphoric acid by the ether-alcohol extraction method, as large an amount of ether-alcohol soluble phosphorus as was obtained in the average samples of the urine examined. This fact points to the conclusion that no ether-alcohol soluble phosphorus is normally present in the urine of rabbits, even after the feeding of organic phosphorus for several months.

A review of this question of the presence of organic phosphorus in the urine in general supports this conclusion. Ronald<sup>a</sup> was the first to call attention to the presence of organic phosphorus in the urine, and Rockwood<sup>b</sup> claims to have found phosphocarnic acid present. Bergmann,<sup>c</sup> however, using glycerophosphoric acid made subcutaneous injections on sheep, but could not detect the same in the urine. Patten, Jordan, and Hart<sup>d</sup> in their extensive experiments with cows found no organic phosphorus eliminated in the urine, and like results were obtained by Mendel and Underhill<sup>e</sup> and by Le Clerc and Cook<sup>f</sup> working with rabbits and a dog.

<sup>a</sup> Philosophical Transactions, 1864, p. 461.

<sup>d</sup> Amer. J. Physiol., 1906, 16:268.

<sup>b</sup> Abs., Chem. Centrbl., 1895 (1), p. 1063.

<sup>e</sup> Ibid., 1906, 17:75.

<sup>c</sup> Arch. exper. Path. Pharm., 1902, 47:77.

<sup>f</sup> J. Biol. Chem., 1906, 2:203.

Mandel and Oertel <sup>a</sup> made some experiments on man along this line, feeding first food poor in phosphorus and later food rich in phosphorus, but found no effect upon the amount of organic phosphorus in the urine. They conclude that the organic phosphorus of the urine is of endogenous origin. The inorganic phosphorus was determined by uranium acetate titration, the solution was boiled with hydrochloric acid and retitrated, the difference being the organic phosphorus.

Organic phosphorus was found in the human urine by Sotnitschewsky.<sup>b</sup> Oertel,<sup>c</sup> using the method of precipitating the inorganic phosphorus with calcium chlorid in ammoniacal solution, found organic phosphorus in the urine of seven men.

Keller <sup>d</sup> undertook some extensive experiments along this line in the case of infants, and he concluded that the food did not influence the amount of organic phosphorus in the urine. He starved himself and found the amount of organic phosphorus eliminated the first three days was constant, while there was an increased elimination the fourth day. This indicated that some highly organic phosphorized tissue—that is, the lymphocytes—were broken down.

Symmers <sup>e</sup> studied this question in various pathological cases—diseases of the nervous system, enteric fever, tuberculosis, diabetes, and lymphatic leucaemia—and found large amounts of organic phosphorus in the urine.

In agreement with most investigators who have studied phytin and its action on the body, Scofone<sup>f</sup> and Giacosa<sup>g</sup> claim that phytin is principally eliminated in inorganic combinations.

There is no doubt that in pathological cases there is considerable organic combined phosphorus eliminated, but there is much doubt as to whether any phosphorus in the organic form is eliminated normally in the urine. The methods which have been employed to separate the two forms of phosphorus are far from satisfactory. The slight difference between two results obtained by different methods, which in many cases would be counted as duplicates, has been classed as due to the presence of organic phosphorus. Moreover, in this work, ether-alcohol soluble phosphorus was found in the urine. This is of little significance, because a solution of sodium hydrogen phosphate yielded an equivalent or greater amount of ether-alcohol soluble phosphorus

<sup>a</sup> N. Y. Univ. Bul. Med. Sci., 1901, 1 : 165.

<sup>b</sup> Zts. physiol. Chem., 1880, 4 : 214.

<sup>c</sup> Ibid., 1898-9, 26 : 123.

<sup>d</sup> Ibid., 1900, 29 : 146.

<sup>e</sup> J. Path. Bact., 1904-5, 10 : 159, 427.

<sup>f</sup> Abs., Biochem. Centrbl., 1905, 3 : 606.

<sup>g</sup> Ibid., 1905, 4 : 572.

by this same method, which precludes the possibility of any phosphorus in this form being normally present in the urine of rabbits.

In Table VI, where the total phosphorus is determined by the Neumann method and the inorganic phosphorus by titration with uranium acetate, the difference is called organic phosphorus. That the uranium acetate method is not absolutely correct is indicated by the figures which in several cases show more phosphorus by this method than by the Neumann method. In the case of rabbit No. 4 and in one instance in the case of rabbit No. 3 the differences are too large to be explained on the basis of experimental error. It is certain from these results that the ingestion of organic phosphorus does not cause an increased elimination of organic phosphorus in the urine; but the fact that in the case of the rabbits fed inorganic phosphorus there should be an apparent elimination of organic phosphorus in the urine in some cases must be explained on the basis of the endogenous origin of the organic phosphorus of the urine which appears to take place only in abnormal cases.

#### CALCIUM AND MAGNESIUM BALANCES.

During the principal metabolism experiments, lasting four weeks, the calcium and magnesium balances were determined in addition to those of nitrogen and phosphoric acid. The amount of calcium ingested per seven-day period varied from 0.44 to 0.58 gram per 1,000 grams body weight, while that of magnesium varied from 0.15 to 0.21 gram, rabbit No. 1 receiving more than rabbits Nos. 3 and 4, while rabbit No. 2, which died at the end of the first two weeks, received about the same amount as rabbits No. 3 and No. 4. Goitein<sup>a</sup> states that unless a rabbit receives 0.16 gram of calcium per kilo body weight, a loss of calcium will occur. The figures show that the rabbits under this observation received far more than that minimum amount and, therefore, were in no danger of calcium starvation.

The figures in Table VII show that the calcium excreted in the urine was 9 per cent for No. 1, 10.8 per cent for No. 2, 4.2 per cent for No. 3, and 4.9 per cent for No. 4. In the case of the rabbits fed organic phosphorus, the average amount of calcium absorbed from the intestinal tract or metabolized was higher than in the case of those fed inorganic phosphorus. These figures agree with the theory that the calcium and phosphorus in the inorganic form unite to form the insoluble calcium phosphate, which is eliminated by the bowels in unchanged form.

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<sup>a</sup> Arch. gesam. Physiol., 1906, 115 : 118.

TABLE VII.—Calcium and magnesium balances—Principal period.

RABBITS FED ORGANIC PHOSPHORUS.

Date.	Rabbit No.	Total ingested.		Excreted in urine.		Excreted in feces.		Total excreted.		Daily balance.		Daily balance ratio.	Excreted in urine.		Absorbed material retained.	
		Ca.	Mg.	Ca.	Mg.	Ca.	Mg.	Ca.	Mg.	Ca.	Mg.		Ca.	Mg.	Ca.	Mg.
1908.																
February 17-23.	1	0.888	0.330	0.029	0.006	0.407	0.139	0.436	0.145	0.065	0.026	1:0.40				
February 24- March 1.....	1	.863	.320	.166	.053	.771	.257	.937	.310	.011	.002	1:0.18				
March 2-8.....	1	.910	.352	.057	.011	.528	.209	.585	.220	.047	.019	1:0.40				
March 9-15.....	1	.910	.352	.068	.013	.551	.244	.619	.257	.049	.014	1:0.29				
Average.....	1	.893	.339	.080	.021	.564	.212	.644	.233	.037	.015	1:0.32	9.0	6.2	75.6	81.8
February 17-23.	2	.858	.279	.118	.119	.363	.158	.481	.277	.053	.000					
February 24- March 1.....	2	.735	.250	.054	.043	.488	.283	.542	.326	.031	-.011					
Average.....	2	.796	.264	.086	.081	.425	.221	.512	.301	.042	-.006					
General av'ge	...	.844	.301	.083	.051	.494	.216	.578	.267	.040	.005					

RABBITS FED INORGANIC PHOSPHORUS.

February 17-23.	3	0.893	0.307	0.046	0.006	0.669	0.223	0.715	0.229	0.025	0.011	1:0.44				
February 24- March 1.....	3	.893	.307	.029	.046	.281	.100	.310	.146	.083	.023	1:0.29				
March 2-8.....	3	.893	.307	.027	.009	.390	.040	.417	.049	.068	.037	1:0.54				
March 9-15.....	3	.893	.307	.049	.008	.133	.056	.182	.064	.102	.035	1:0.34				
Average.....	...	.893	.307	.038	.017	.368	.105	.368	.122	.069	.027	1:0.40	4.2	5.5	94.6	91.5
February 17-23.	4	.788	.258	.020	.005	.285	.098	.305	.103	.069	.022	1:0.32				
February 24- March 1.....	4	.893	.307	.050	.015	.261	.063	.311	.078	.083	.033	1:0.40				
March 2-8.....	4	.893	.307	.060	.021	.419	.106	.479	.127	.059	.026	1:0.44				
March 9-15.....	4	.893	.307	.043	.025	.394	.096	.431	.121	.065	.026	1:0.40				
Average.....	...	.867	.296	.043	.017	.339	.091	.382	.107	.069	.027	1:0.39	4.9	5.7	91.9	91.7
General av'ge	...	.880	.302	.041	.017	.354	.095	.376	.115	.069	.027	1:0.40	4.6	5.6	93.3	91.6

In the case of man, from 5 to 10 per cent of the calcium is normally excreted in the urine; the remainder is excreted with the feces either directly, or a part may be absorbed from the small intestine and excreted into the large intestine, as shown by Voit<sup>a</sup> in the case of the dog. Some calcium may come from the intestinal juices. According to Schetelig,<sup>b</sup> Von Noorden,<sup>c</sup> and Rumpf,<sup>d</sup> the amount of calcium excreted in the urine increases with the amount of water taken into the system. Various authors have increased the calcium elimination by adding acids (especially hydrochloric acid) and salts to the food. Hoppe-Seyler<sup>e</sup> and Von Noorden found an increased calcium elimination during complete rest. A calcium retention was found by Rumpf, Hirschler, and Terray<sup>f</sup> in feeding milk (containing 1.58 grams of calcium per liter). The total volume of urine in each case

<sup>a</sup> Cited, Hammarsten, Textbook of Physiological Chemistry, rev. ed., New York, 1908.

<sup>b</sup> Virchow's Archiv, 1880, 82:437.

<sup>c</sup> Beitr. Lehre Stoffwechsel gesund. krank. Menschen, Berlin, 1902.

<sup>d</sup> Berlin. klin. Wochenschr., 1897, 34:265.

<sup>e</sup> Zts. physiol. Chem., 1891, 15:161.

<sup>f</sup> Zts. klin. Med., 1905, 57:137.

was practically the same, varying only from 975 to 1,100 cc per period on an average, and yet the calcium excreted was subject to considerable variation even in the case of the same rabbit fed on the same diet for several weeks. This fact is contrary to the findings of Patterson,<sup>a</sup> who found that on a fixed diet with man the urinary calcium ran parallel to the total amount of urine excreted. Although there was a smaller amount of calcium metabolized by the rabbits fed inorganic phosphorus, yet of this amount a larger proportion was retained than in the case of those fed organic phosphorus.

By excluding the very abnormally high amount of magnesium found in the urine in the case of rabbit No. 2 (which died), we find a very close agreement in the case of the other three animals, though there is a slight tendency for the rabbit fed organic phosphorus to excrete more magnesium in the urine. The amount of metabolized magnesium that was retained shows that the rabbits fed inorganic phosphorus, while metabolizing a smaller amount of the magnesium than did those fed organic phosphorus, retained a larger per cent of the amount actually metabolized. In man from 29 to 38 per cent of the ingested magnesium is excreted in the urine, which is higher than in the case of rabbits. The ratio of calcium to magnesium eliminated in the urine is not constant in the cases studied. According to Bertram<sup>b</sup> and Renvall,<sup>c</sup> 29 to 38 per cent of magnesium is excreted in the urine and 62 to 71 per cent is eliminated in the feces. It is more easily excreted through the kidneys than is calcium. The ratio of calcium oxid to magnesium oxid excreted in human urine, according to Klemperer and Tritschler,<sup>d</sup> varies from 1:0.8 to 1:1.2. The ratio of calcium to magnesium excreted in the feces in the cases of rabbits Nos. 1 and 2 is lower than in the case of Nos. 3 and 4. The ratio of calcium to magnesium excreted by man is held to be 8:1, but in the case of the rabbits the ratio is considerably lower. The amount of magnesium required by man is placed at 0.6 gram per day. In all cases of the rabbits experimented with, daily positive calcium and magnesium balances were obtained.

#### CHEMICAL ANALYSIS OF THE BODIES OF THE RABBITS.

In all cases the analyses were made on composite samples of two rabbits, and represent the average figures. All analyses were calculated to a water-free basis.

#### BONES.

The bones were freed from the adhering muscular and tendon tissue and placed in a large kettle and boiled for several hours with water until all the flesh could be easily removed. They were then dried in a hot-air bath, again scraped, and finally ground into a fine powder. The bone powder in the case of the two normal rabbits was

<sup>a</sup> Bio-Chem. J., 1908, 3:39.

<sup>b</sup> Abs. Chem. Centrbl., 1897, 68:957.

<sup>c</sup> Skand. Arch. Physiol., 1904, 16:94

<sup>d</sup> Zts. klin. Med., 1902, 44:337.



lighter in color and did not have the oily feeling characteristic of that of the phosphorus-fed rabbits. The water content of the normal bones was lower than in the cases where phosphorus was fed. This was due to the higher fat content in those cases. The remainder of the figures represent the results calculated to a water-free basis, and are shown in Table VIII.

TABLE VIII.—*Chemical analyses of bodies of rabbits (water-free basis).*

## RABBITS FED ORGANIC PHOSPHORUS, NOS. 1 AND 2.

Description of sample.	Nitrogen.	Ash.	Calcium.	Magnesium.	Ether extract.	Phosphoric acid.		
						Total.	Ether-alcohol soluble.	Ether-alcohol soluble in terms of total.
						Per cent.	Per cent.	Per cent.
Bones.....	4.53	55.56	8.86	0.22	11.33	23.96	0.055	0.23
Livers.....	7.64	3.78	.00	.00	44.95	1.98	.680	34.34
Blood.....	14.72	5.64	Trace.	.00	Trace.	.75	.008	1.07
Brains.....	6.12	7.00	.31	.09	43.23	3.96	2.350	59.34
Nerves.....	5.52	6.54	.21	.11	46.96	3.72	2.390	64.26
Teeth.....		74.65	24.73	1.35		35.31		

## RABBITS FED INORGANIC PHOSPHORUS, NOS. 3 AND 4.

Bones.....	4.68	55.92	7.74	0.15	9.39	26.33	0.061	0.23
Livers.....	9.41	4.73	.00	.00	34.48	2.56	.854	33.36
Blood.....	14.42	4.72	Trace.	.00	Trace.	.69	.008	1.16
Brains.....	6.32	7.17	.27	.06	43.89	4.07	1.160	28.51
Nerves.....	3.72	5.83	.28	.06	47.34	4.28	1.470	34.35
Teeth.....		76.10	24.65	1.22		34.70		

## NORMALLY FED RABBITS, NOS. 5 AND 6.

Bones.....	4.32	57.47	10.17	0.23	5.12	25.93	0.069	0.27
Livers.....	11.90	5.67	.00	.00	14.47	2.85	1.060	38.24
Blood.....	14.42	4.55	.44	.19	Trace.	.58	.037	6.33
Brains.....	6.85	7.82	.51	.08	38.50	4.16	1.750	42.07
Nerves.....	4.39	5.97	.44	.12	44.34	3.90	2.310	59.23
Teeth.....		75.17	28.35	1.15		35.63		

The normal rabbits show a slightly higher figure for total ash in the bones than do the phosphorus-fed rabbits. Several investigators have pointed out the fact that the skeleton becomes poorer in water and richer in ash with age. Voit<sup>a</sup> showed this fact in the case of dogs, and Brubacher<sup>b</sup> in the case of children. There is a considerable variation in the ratio of the calcium to the total ash, even in normal animals. Wellmann<sup>c</sup> states that in cases of starved rabbits the bones show a smaller percentage of organic matter, that is, a higher ratio of ash. In the case of the normal rabbits, where the highest ash is present, we expect to find the highest percentage of ash constituents, and such is the case.

<sup>a</sup> Cited, Hammarsten, Textbook of Physiological Chemistry, rev. ed., New York, 1908.

<sup>b</sup> Zts. Biol., 1890, 27 : 517.

<sup>c</sup> Arch. gesam. Physiol., 1908, 121 : 508.

The amounts of ether-soluble matter present in the bones show some very interesting results. Both when organic and inorganic phosphorus were fed, the bones contained more ether-soluble matter than the normal bones, evidently a case similar to the increased percentage of ether-soluble material in the liver and presumably due to the phosphorus fed. The bones of the normal rabbits and of those fed inorganic phosphorus show practically an equal amount of total phosphorus, the figure for the rabbits fed organic phosphorus being a little lower. The bones of the normally fed rabbits contain a slightly higher percentage of ether-alcohol soluble phosphorus than in the other cases. The conclusion is that feeding this large amount of phosphorus has not materially affected the quantity of phosphorus stored in the bones, nor has the form of that storage been appreciably changed. There is 0.27 per cent of the total phosphorus as ether-alcohol soluble phosphorus in the bones of the normal rabbits, and 0.23 per cent in the other cases, but the difference, 0.04 per cent, is not large enough to have any significance. In a matter of this kind the age of the animal should be taken into account, for it is known that there is less calcium phosphate and more carbonate in the bones of old rabbits than in the bones of young ones; likewise, less calcium, magnesium, and phosphorus in the bones of starved rabbits, as shown by Wellmann.

On a fat-free, water-free basis the analysis of the bones; given in Table IX, shows a slight variation in the ash content, the ash of the rabbits fed organic phosphorus being slightly higher than that for the bones of the rabbits fed inorganic phosphorus, which in turn is higher than the normal. In regard to the calcium and magnesium, the bones of the rabbits fed inorganic phosphorus contain the smallest amount, while the highest percentage of calcium is present in the normal bones. The total ash content in the cases of the experimental rabbits was greater than in the normal rabbits. The bones of the rabbits fed inorganic phosphorus show a higher phosphorus content than in the other cases; but the results as regards ether-alcohol soluble phosphorus show little variation, the bones of the normal rabbits containing the largest proportion and those of the rabbits fed organic phosphorus the smallest proportion.

TABLE IX.—*Analysis of bones calculated to a fat- and water-free basis.*

Rabbits.	Calcium.	Magnesium.	Ash.	Phosphoric acid.			
				Total.	Ether-alcohol soluble.	Ether-alcohol insoluble.	Ether-alcohol soluble in terms of total.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Fed organic phosphorus.....	9.99	0.25	62.66	27.04	0.065	26.97	0.26
Fed inorganic phosphorus.....	8.54	.17	61.71	29.06	.067	28.99	.23
Normal.....	10.72	.24	60.57	27.33	.073	27.26	.28

## LIVERS.

The livers of the rabbits were dried and powdered. The total ash of the livers of the normal rabbits is higher than the ash of the livers of those fed inorganic phosphorus which, in turn, is slightly higher than the ash of the livers of those fed organic phosphorus.

The changes produced in the livers do not seem to include any large storage of phosphorus in the organ, for the livers of the rabbits fed organic phosphorus which show the largest excess of fat contain the lowest percentage of total and ether-alcohol-soluble phosphorus, and the livers of the rabbits fed inorganic phosphorus, which have a lower fat content, contain a lower percentage of phosphorus than the normal rabbits' livers. Not only is there a difference in the total phosphorus content of the various livers, but the livers of the rabbits fed organic phosphorus contain a lower percentage of ether-alcohol-soluble phosphorus than is present in the other cases, the normal livers showing the highest figures. That there is an excess of fat in the livers of the rabbits fed an excessive amount of phosphorus, whether organic or inorganic, is shown by the figures for the ether-soluble matter, namely, 44.95 per cent in the case of the rabbits fed organic phosphorus, 34.48 per cent in the livers of those fed inorganic phosphorus, and only 14.47 per cent in the case of the livers of the normally fed rabbits.

TABLE X.—*Analysis of rabbits' livers (water-free basis).*

No. of rabbit.	Weight of rabbit.	Ration.	Weight of liver.		Percentage composition.			Actual amounts.		Calculated to a fat-free basis.			
			Moist.	Dry.	Nitrogen.	Phosphoric acid.	Ether extract.	Total nitrogen.	Total phosphoric acid.	Nitrogen.		Phosphoric acid.	
										Total.	Per 1,000 grams body weight.	Total.	Per 1,000 grams body weight.
1.....	Gms. 1,550	Organic phosphorus	Gms.	Gms.	P. ct.	P. ct.	P. ct.	Gms.	Gm.	Gms.	Gms.	Gms.	Gm.
3.....	1,800		Inorganic phosphorus	91.0	36.1	7.64	1.98	44.95	2.76	0.72	5.01	3.23	1.31
4.....	2,000	do	81.5	27.5	9.41	2.56	34.48	2.58	.70	3.94	2.18	1.07	.60
5.....	2,050	Normal	86.0	35.0	9.41	2.56	34.48	3.29	.90	5.02	2.50	1.37	.69
6.....	2,200	do	59.0	14.6	11.90	2.85	14.47	1.74	.42	2.04	1.00	.49	.24
			60.0	15.0	11.90	2.85	14.47	1.79	.43	2.09	.95	.50	.23

In Table X the results for the body weights, liver weights, total nitrogen, total phosphorus, and ether extract found in the livers of the various rabbits are given. That phosphorus in the forms fed tends to enlarge the livers is seen from the percentage of total weight of the livers in terms of the body weight of the rabbits. In the case of Nos.

5 and 6 (the normal rabbits) the livers formed 3 and 2.8 per cent, respectively, of the total weight of the rabbits, while in Nos. 1, 3, and 4 the liver weight made 6.0, 4.5, and 4.4 per cent of the body weight. More total nitrogen and phosphorus is present in the livers of phosphorus-fed rabbits than in the normal livers, and calculated to a fat-free basis the differences are more evident. The tendency of organic phosphorus to produce fat is especially striking; in these experiments an increased amount of ether-soluble matter is seen in the bones, livers, brains, and nerves of the phosphorus-fed rabbits. Jordan, Patten, and Hart,<sup>a</sup> in their experiments with phytin, state that when the cows were reduced from a high to a low phytin diet, the percentage of fat in the milk was reduced. The fact that phytin tends to the production of fat in various organs and secretions seems to be established by all of these experiments.

#### BLOOD.

The figures for blood analysis are given on a water-free basis. The amount of ash is highest in the blood of the rabbits fed organic phosphorus. The blood of those fed inorganic phosphorus and of the normal rabbits contain about an equal amount of ash. The variations are not large enough to be of any consequence when the individuality of the different rabbits is considered. The calcium content of the blood of all the phosphorus-fed rabbits is reduced, showing only a trace, while 0.44 per cent of calcium is present in the dried blood of the normal rabbits. The same holds true in the case of the magnesium, 0.19 per cent being present in the dried blood of the normal rabbits and none being found in the blood of the phosphorus-fed rabbits. The prevailing belief is that the composition of the blood changes but little if any, no matter what changes may take place in the body tissues. Patterson<sup>b</sup> found, in the case of animals kept on a calcium-poor diet, that the ratio of the calcium of the blood to the total ash of the blood was the same as that of normal animals. The amount of total phosphorus present in the normal blood is somewhat lower than in the cases where phosphorus was fed. The ether-alcohol soluble (lecithin) phosphorus is highest in the blood of the normal rabbits and the figures show that 6.33 per cent of the total phosphorus is present in the dried blood of the normal rabbits as ether-alcohol soluble phosphorus against 1.07 and 1.16 per cent in the case of the experimental rabbits. Here again we see that the constant ratio of the constituents of the blood is not maintained. Only traces of ether-soluble matter were found in any of the samples of blood examined. (Table VIII.)

<sup>a</sup> Amer. J. Physiol., 1906, 16 : 268.

<sup>b</sup> Bio-Chem. J., 1908, 3 : 39.

## BRAINS.

The brains of the rabbits were completely removed and dried in platinum dishes, then ground in a mortar and mixed as well as possible before analyzing. The ash varied from 7.0 to 7.82 per cent, being highest in the normal brains. As the age and other factors influence the ash content of all organs, this variation has no significance. The percentage of calcium shows some variation and is likewise highest in the ash of the brains of the normal rabbits.

The percentage of magnesium remains fairly constant in all cases. The ratio of calcium to magnesium in the brains is 6.3:1 in the normal rabbits, 4.5:1 in the brains of those fed inorganic phosphorus, and 3.5:1 in the brains of those fed organic phosphorus.

The total phosphorus found in the brains is about the same in all cases, but this is not true of the ether-alcohol soluble phosphorus. The brains of the rabbits fed organic phosphorus show 2.35 per cent phosphorus as ether-alcohol soluble, or 59.34 per cent of the total. The brains of those fed inorganic phosphorus show but 1.16 per cent phosphorus as ether-alcohol soluble phosphorus, or 28.51 per cent of the total. This is lower than the figures for brains of the normal rabbits, which contain 42.07 per cent of the phosphorus in a form soluble in ether and alcohol. It appears from these figures that the brains of the rabbits fed organic phosphorus contain an appreciably larger amount of ether-alcohol soluble phosphorus than do the brains of rabbits fed on inorganic phosphorus or those of the normally fed rabbits. (Table VIII.)

## NERVES.

The ash content on a water-free basis shows a fairly constant percentage in all cases. As in the brain, the calcium content of the nerves is higher for the normal rabbits than in the other cases.

The amount of magnesium in the nerves of the normal rabbits is 0.12 per cent, 0.11 per cent, or practically the same, in the nerves of those fed organic phosphorus, and one-half, or 0.06 per cent in the nerves of those fed inorganic phosphorus.

The ratio of the calcium to the magnesium is much lower in the nerves of the rabbits fed organic phosphorus than in the other cases. The data for ether-soluble material are fairly uniform, being a trifle lower for the nerves of the normal rabbits than for the experimental rabbits.

The total phosphorus content varies from 3.72 per cent in the case of the rabbits fed organic phosphorus to 4.28 per cent in the case of those fed inorganic phosphorus. The amount of ether-alcohol soluble phosphorus is lowest in the case of the rabbits fed on inorganic phosphorus, the percentage of the total phosphorus in this form being

34.25 per cent against 64.26 per cent in the case of the rabbits fed on organic phosphorus, and 59.23 per cent in the normal rabbits. These results are similar to those recorded in the case of the brains. (Table VIII.)

#### TEETH.

The teeth were freed from adhering bone and muscle tissue by scraping and were dried in a hot-air oven at 100° C. The ash was constant in all cases, averaging 75 per cent. There was somewhat more calcium and a trifle less magnesium found in the teeth of the normal rabbits than in the teeth of the phosphorus-fed rabbits. These results run parallel with those obtained for calcium in the case of the bones of the rabbits. The total phosphorus was practically no higher in the normal rabbits than in the other cases. (Table VIII.)

#### INTESTINES.

Phosphorus was estimated in portions of the small intestines of the various rabbits. The loops of intestine were well washed and dried between filter papers and then in an air bath at 45° C. for five hours. When sufficiently dry they were cut into small bits. Moisture and total phosphorus were determined on a weighed quantity of this substance and on another portion the following extractions were made:

One gram of substance was ground fine with pure sand in a porcelain mortar and transferred to a 300 cc Erlenmeyer flask. Thirty cubic centimeters of absolute ether were then added and the whole extracted on the water bath overnight, using a reflux condenser. The ether extract was then filtered through a hardened filter into an ordinary Jena flat-bottom flask. Particles of residue found on the filter paper were scraped back into the Erlenmeyer flask. To this ether extract residue 60 cc of absolute alcohol were added and boiled for three hours, using a reflux condenser. This alcohol extract was filtered hot into the Jena flask containing the ether extract and the residue washed twice with separate portions of 25 cc of hot alcohol and the washings were added to the combined extract. Phosphorus was determined in the combined ether-alcohol filtrate by the Neumann method.

The ether-alcohol extraction residue was next treated six times with 50 cc portions of cold water saturated with chloroform, allowing ten hours for each extraction, using the same hardened filter paper as before and scraping back the residue from the filter paper into the flask. This solution was evaporated to dryness and the phosphoric acid determined therein by the Neumann method. This is called the phosphorus insoluble in ether and alcohol, but soluble in water. Phosphoric acid was then obtained in the residue by difference and called the phosphorus insoluble in ether, alcohol, and water.

In all cases a large amount of the phosphorus of the fresh sample was soluble in water, fully 50 per cent being dissolved by this means during the preparation of the sample. The amount of phosphorus insoluble in alcohol, ether, and water was higher in the normal rabbits' intestines than in the other cases. The amount of ether-alcohol soluble phosphorus showed but little variation.

## SUMMARY.

There are many variations in the composition of the different portions of the rabbits, apparently due to the feeding of phosphorus in the two forms. The amount of ether-alcohol-soluble phosphorus stored in the brains and nerves is much lower in the case of the rabbits fed inorganic phosphorus than in the normal rabbits and those fed organic phosphorus. The organic and the inorganic phosphorus, fed in excess, caused an increased percentage of ether-soluble material in the bones, and an indication of the same is noted in the brains and in the nerves. No increased storage of calcium or magnesium was noted; on the contrary there is a slight decrease, as compared with the normal, in bones, nerves, and brain. The ether-alcohol soluble phosphorus of the blood was reduced in all cases where phosphorus was fed. The increase in the fat of the livers was marked in both cases of phosphorus feeding, but was greater for the rabbits fed organic phosphorus. If a large percentage of ether-alcohol soluble phosphorus in the brains and nerves is desirable, then the rabbits fed organic phosphorus were in a better condition, according to the data, than were those fed on inorganic phosphorus.

The nitrogen content was determined in all of the samples, and is given in Table VIII calculated to a water-free basis. The percentage of nitrogen found in the bones varied from 4.32 per cent in the bones of the normal rabbits to 4.68 per cent in the bones of the rabbits fed on inorganic phosphorus. The percentage of nitrogen found in the livers of the experimental rabbits was considerably lower than in the livers of the normal rabbits, this agreeing with the other data showing a generally poor condition of the livers of the former. The percentage of nitrogen found in the blood was fairly constant, as is usually the case, and the same is true of the brains. Just why we find a variation in the amount of nitrogen present in the nerves and spinal cord is difficult to explain. More ash and more ether-alcohol-soluble phosphorus are found in the samples of nerves of the rabbits fed organic phosphorus, which also show the highest percentage of nitrogen. The ratio of phosphorus to nitrogen in the liver of rabbits is 1:14.7, according to Wellmann.<sup>a</sup> His figures are higher than the results obtained in this experiment, the ratio of phosphorus to nitrogen being, for normal rabbits 1:9.7, for rabbits fed organic phosphorus 1:8.8, and for those fed inorganic phosphorus 1:8.6.

<sup>a</sup> Arch. gesam. Physiol., 1908, 121:508.

## FINDINGS OF AUTOPSIES.

At the conclusion of the principal feeding period the rabbits were chloroformed, autopsies were made, and histological slides of several of the organs prepared. Two normal rabbits were similarly treated. The normal rabbits had been fed on corn, oats, and vegetables for some time previously and had been kept in cages. There seems to be no constant relationship between the total weights and the percentages of solids present in the various organs, blood, brains, and nerves of the six rabbits examined. The autopsies showed the following results: <sup>a</sup>

*Rabbit No. 1, fed organic phosphorus.*

Intestines: Normal, containing food. Some fat distributed along the intestines.  
 Lymphatics: Apparently normal.  
 Kidneys: Apparently normal.  
 Spleen: Apparently normal.  
 Liver: Very light in color, possibly fatty infiltration. Appearance similar to that of No. 2.  
 Stomach: Contained food. Normal.  
 Heart: Normal.  
 Lungs: Showed a condition of anemia along edges.  
 After drying: Bones seemed oily. Much fat in liver; fat left in bottom of dish after drying.

*Rabbit No. 2, fed organic phosphorus.*

Intestines: Normal, except colon distended with large amount of feces; no congestion.  
 Lymphatics: Apparently normal.  
 Kidneys: Apparently normal.  
 Spleen: Apparently normal.  
 Liver: Somewhat enlarged, pale, with yellow tinge; seemed pathological.  
 Stomach: Full of food; appeared to have extended area of old hemorrhage on lesser curvature.  
 Heart: Apparently normal; post-mortem blood clot.  
 Lungs: Left, apparently normal; right, partly congested.  
 Nervous system: Normal.  
 General appearance: Good.

*Rabbit No. 3, fed inorganic phosphorus.*

Intestines: Apparently normal.  
 Lymphatics: Apparently normal.  
 Kidneys: There seemed to be slight irritation and congestion.  
 Liver: Light colored; enlarged.  
 Spleen: Normal in size and color generally; better condition than Nos. 1 and 2; yellow color; numerous small areas of what may be fatty degeneration or infiltration.  
 Stomach: Old hemorrhage around greater curvature.  
 Heart: Apparently normal.  
 Lungs: General appearance good, but apex of right lung had a small area of congestion.  
 General appearance: Fairly fat and in good condition externally.

<sup>a</sup>H. L. Amoss, of the Animal Physiological Laboratory, assisted in making the autopsies and interpreting the histological slides which were prepared by E. A. Read, of the Microchemical Laboratory.



*Rabbit No. 4, fed inorganic phosphorus.*

Intestines: Apparently normal.

Kidneys: Right, slight yellow color in cortex, pitted, congested; left, pitted over external surface, slight congestion in medulla, very slight yellowish color in cortex.

Spleen: Apparently normal.

Liver: Light color, lighter spots seen. Gall bladder apparently normal.

Stomach: Capillaries of fundus darkened—apparently not post-mortem change.

Heart: Apparently normal.

Lungs: Left, apparently normal; right, lower half of lower lobe congested.

*Rabbits Nos. 5 and 6, normally fed.*

The autopsies showed that in the cases of the two normal rabbits, Nos. 5 and 6, the organs were apparently normal. The livers had the normal dark-red color in contrast with the pale livers of Nos. 1, 2, 3, and 4.

The autopsies showed that none of the experimental rabbits was in a normal condition. The livers were especially affected, being pale and abnormal, and indicating an excess of fat.

The relation of the liver to metabolism is a problem important to both the physiologist and the pathologist. For a long time it has been recognized that one of the functions of the liver is connected in some way with the destruction or removal of poison from the blood, especially such poisons as are produced in, or absorbed from, the alimentary canal. It was early recognized that the destruction of the liver cells leads to serious poisoning, and this was experimentally demonstrated by Eck, who excluded the liver from the circulation by making a direct communication between the portal vein and the inferior vena cava, an operation known as "Eck fistula."

The question of fatty degeneration has an important bearing from the physiological point of view, for fatty degeneration is another proof of the formation of fat from proteins. From the investigations of Bauer<sup>a</sup> on dogs and Leo<sup>b</sup> on frogs we must admit that at least in acute poisoning by phosphorus fatty degeneration takes place with the formation of fats from proteins. Still, investigators are not unanimous on this point, and Pflüger<sup>c</sup> especially has raised objections to these experiments.

The ideas of the fatty degeneration of protein in the old sense as used by these writers have been changed by the work of Rosenfeld,<sup>d</sup> etc., who believe in the theory of fat transportation.

In the case of a phosphorized dog, Mandel<sup>e</sup> has shown that lactic acid disappears from the blood and urine when phlorhizin glycosuria

<sup>a</sup> Zts. Biol., 1871, 7 : 63.

<sup>b</sup> Zts. physiol. Chem., 1885, 9 : 469.

<sup>c</sup> Arch. gesam. Physiol., 1891-92, 51: 317.

<sup>d</sup> Ergeb. Physiol., Pt. I, Biochemie, 1903, 2 : 50.

<sup>e</sup> Amer. J. Physiol., Proc., 1905, 13 : 16.

is induced. Lusk<sup>a</sup> offers the following general hypothesis as his explanation of fatty changes in tissues:

The lactic acid which occurs is derived from the sugar formed in proteid metabolism. In the above case the sugar is removed before its conversion into lactic acid. In phlorhizin diabetes, dextrose does not burn; in phosphorus poisoning lactic acid derived from dextrose does not burn. In both cases a sugar-hungry cell, or one where carbohydrate is not oxidized, is found, and under these circumstances fat is attracted to the cell, and in larger quantities than can be useful. Wherever sugar freely burns this fatty infiltration is impossible. A reduced local circulation in a portion of the heart may produce anemia of the part, an imperfect local combustion of lactic acid normally formed and a fatty infiltration of the locality.

\*                    \*                    \*                    \*                    \*                    \*                    \*

It has been stated that the action of phosphorus is to induce autolysis (self-digestion) of the body's protoplasm (Jacoby,<sup>b</sup> Waldvogel<sup>c</sup>), since leucin, tyrosin, and other amino acids may be eliminated in considerable quantity in the urine. Oswald<sup>d</sup> thinks that phosphorus destroys or weakens the antiautolytic agents of the body. That autolytic enzymes do not gain free control over the cells through the direct influence of phosphorus is proved by the work of Ray, McDermott, and Lusk.<sup>e</sup> These authors found that phosphorus injections raised the proteid metabolism of fasting dogs to 250, 260, 283, 248, 183, and 164 per cent of that of the dog when normal. They contrasted this increased proteid metabolism with that obtained in phlorhizin glycosuria, which is represented by increases to 540, 450, 340, and 340 per cent. When, however, they gave phlorhizin and obtained the increased metabolism, and then injected phosphorus, this was not followed by any marked increase in proteid metabolism. Under these circumstances phlorhizin glycosuria is the predominating factor, removing the dextrose produced from proteid. As regards phosphorus poisoning, Araki<sup>f</sup> believes that lactic acid accumulation is due to lack of oxygenation of the tissues caused by a slow heart beat, but not due to anemia. He does not believe the oxygen deprivation to be very pronounced. The writer offers the explanation that phosphorus may affect the enzyme which breaks up the lactic acid derived from dextrose, and the accumulation of this acid may prevent the action of some of the denitrogenizing enzymes; and further, its noncombustion may necessitate an increase of proteid metabolism.

This theory is strengthened by the discovery of Schryver<sup>g</sup> that the addition of lactic acid favors the accumulation of amino acids in autolysis of the liver.

In this connection we must recognize the fact that the presence of amino bodies—leucin, tyrosin, etc.—in the liver in cases of phosphorus poisoning is well established. Abderhalden and Bergell<sup>h</sup> detected glycocoll in the urine of a rabbit poisoned with phosphorus. Wohlgemut<sup>i</sup> found phenylanin and arginin in the urine after a case of phosphorus poisoning. An altered quantitative relationship between

<sup>a</sup> The Elements of the Science of Nutrition, Philadelphia, 1906.

<sup>b</sup> Zts. physiol. Chem., 1900, 30 : 174.

<sup>c</sup> Arch. klin. Med., 1905, 82 : 437.

<sup>d</sup> Biochem. Centrbl., 1905, 3 : 365.

<sup>e</sup> Amer. J. Physiol., 1899, 3 : 139.

<sup>f</sup> Zts. physiol. Chem., 1893, 17 : 310.

<sup>g</sup> Bio-Chem. J., 1906, 1 : 123.

<sup>h</sup> Zts. physiol. Chem., 1903, 39 : 464.

<sup>i</sup> Ibid., 1905, 44 : 74.

arginin, lysin, and histidin was noted in the liver of a phosphorus-poisoned dog by Wakeman.<sup>a</sup> The amount of arginin was considerably lower than the amount present in the liver of a normal dog.

In the experiments here reported there is no doubt that, due to organic and inorganic phosphorus, an alteration in the livers, brains, and nerves has taken place, accompanied by the presence of abnormally high percentages of fat.

#### HISTOLOGICAL EXAMINATION.

The following results were obtained from the histological examination:

Rabbits Nos. 1 and 2, fed organic phosphorus.

Liver, No. 1: Extensive areas of fatty degeneration and fatty infiltration. Sections stained with hemotoxylin and eosin, Fleming's solution, and Soudan III.

Liver, No. 2: Not quite as extensive areas of fatty degeneration and fatty infiltration as in the case of No. 1. Chronic inflammatory processes seen and general areas of hemorrhages.

Lungs, No. 1: Several areas of focal necrosis noticed surrounding vein in a state of passive congestion. Practically all vesicles collapsed and walls congested. General red cell extravasation.

Stomach, No. 2: Very slight congestion.

Rabbits Nos. 3 and 4, fed inorganic phosphorus.

Liver, No. 3: Infiltration in certain areas, but not as far gone as Nos. 1 and 2. Certain areas showed little change. Slight cloudy swelling. Slight chronic inflammation, not so marked as No. 2.

Liver, No. 4: Little difference compared with Nos. 1 and 2. Some subacute inflammatory processes seen, also some fatty degeneration. Soudan III stain shows more fatty degeneration than is seen with Fleming's.

Lungs, No. 3: Pneumonia in stage of gray hepatization.

Lungs, No. 4: Not normal, but in better general condition than others. Lumen of artery nearly obliterated by fibrous tissue. Bronchitis. Slight general congestion.

Heart, No. 3: Pericarditis. Extensive fatty degeneration of myocardium.

Kidneys, No. 3: Marked congestion. Slight parenchymatous degeneration.

Kidneys, No. 4: Parenchymatous degeneration. Congestion more pronounced than in case of No. 3.

Stomach, No. 3: Apparently normal.

Stomach, No. 4: Apparently normal.

Rabbits Nos. 5 and 6, normally fed.

Livers: Practically normal. No fat present; small areas in which few nuclei did not stain as deeply as the rest, probably due to sectioning.

Lungs: Congestion throughout. A few areas of hemorrhages with superimposed inflammatory processes.

Kidneys: Slight intertubular hemorrhage.

Hearts: Slight myocarditis.

Stomachs: Apparently normal.

The results of the histological examination confirm the opinions formed at the time of the autopsy and show most marked fatty

<sup>a</sup> Zts. physiol. Chem., 1905, 44 : 335.

degeneration of the livers of rabbits Nos. 1 and 2. The liver of rabbit No. 4 also shows fatty degeneration, but less marked, while rabbits Nos. 5 and 6 show normal livers. That the harmful effects noted are due to the excessive amounts of phosphorus fed either organic or inorganic, is proven from the cases of the normal rabbits which, although likewise kept in cages, showed apparently normal livers. In reviewing the phosphorus literature it was noticed that in several cases rabbits fed on organic phosphorus were reported to have died of pneumonia, as did rabbit No. 2 in the experiments here recorded.

Six photomicrographs<sup>a</sup> are appended, which show sections of the livers of rabbits Nos. 1, 2, and 4. The tissue in the case of rabbit No. 3 was exhausted before an osmic acid slide was prepared and it was therefore impossible to give a reproduction in this case. The photomicrographs in which the fat is shown stained black in position demonstrate that degeneration as well as fatty infiltration has taken place in the livers of both of the organic and in one of the inorganic-phosphorus-fed rabbits.

The author is indebted to Doctor Mohler, of the Bureau of Animal Industry, for his interpretation of the microscopic slides, in regard to which he makes the following statement:

The section in the case of rabbit No. 1 (Pl. I) shows a more marked and advanced lesion of fatty degeneration than do the three remaining cases. In this case fatty infiltration is also present and occurs principally on the periphery of the lobules and in the tract supplied by branches of the portal vein, while the fatty degeneration is more abundant in the central zone around the hepatic vein. In fatty degeneration the fat is usually in minute granules, which may coalesce to form small droplets, but only in the most advanced stages do they form large drops as is the case in fatty infiltration.

The sections in which the tissue has been treated with Fleming's solution show the lesion best, as the contrast of the black-stained fat is so marked as compared with the light hepatic parenchyma that photomicrographs may be readily made. The Soudan III sections, while valuable for demonstration purposes, can not be reproduced as well on account of less contrast and the red color.

The section in the case of rabbit No. 4 (Pl. III) shows a less advanced stage of fatty degeneration; there is also not much fatty infiltration present. In the section in the case of No. 2 (Pl. II) there is still less fatty degeneration present, while the section of rabbit No. 3 does not show any degeneration and only a little fatty infiltration.

### CONCLUSIONS.

The somewhat limited experimental data here reported point to the following conclusions, which may be altered by more extensive work on the subject.

#### PRELIMINARY FEEDING PERIOD.

During the preliminary feeding period, the rabbits fed on organic phosphorus excreted a slightly larger proportion of nitrogen in the urine than did the rabbits fed on inorganic phosphates, but retained

<sup>a</sup> Made by B. J. Howard, Chief, Microchemical Laboratory, Bureau of Chemistry.

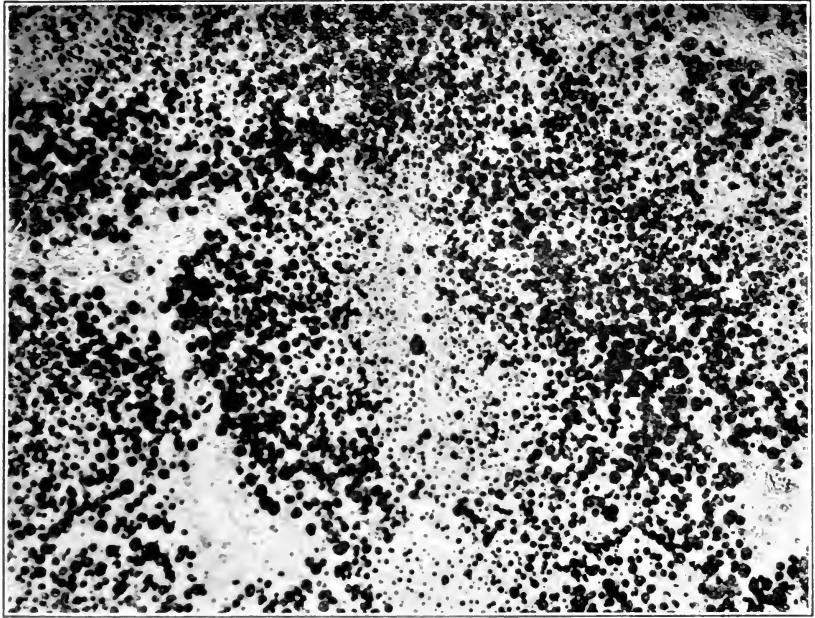


FIG. 1.—MAGNIFICATION 60 DIAMETERS.

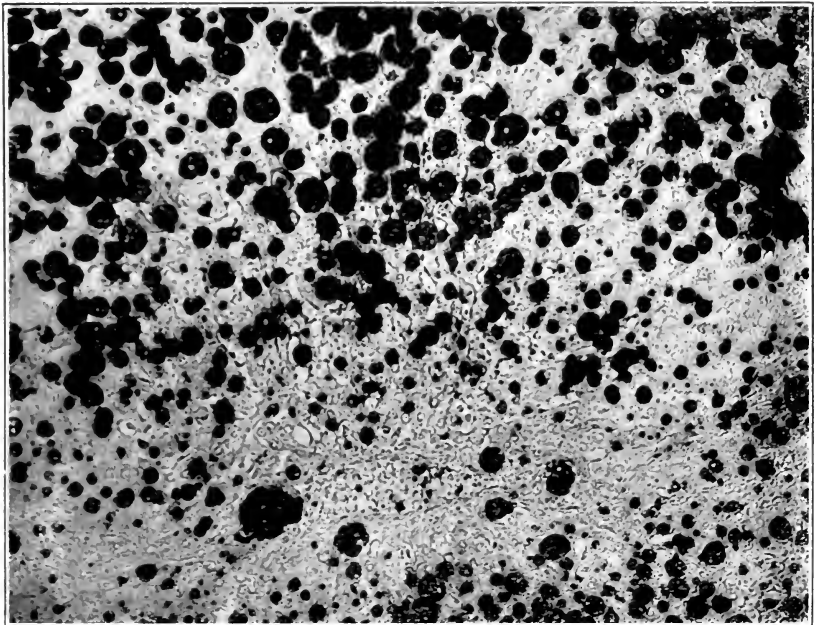


FIG. 2.—MAGNIFICATION 175 DIAMETERS.

LIVER SECTIONS OF ORGANIC-PHOSPHORUS-FED RABBIT NO. 1.  
Fat stained black with osmic acid.



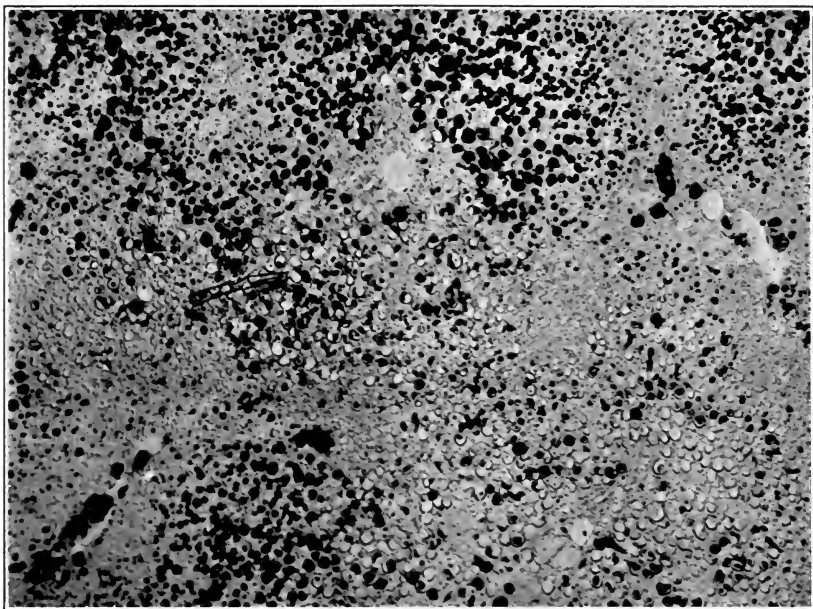


FIG. 1.—MAGNIFICATION 60 DIAMETERS.

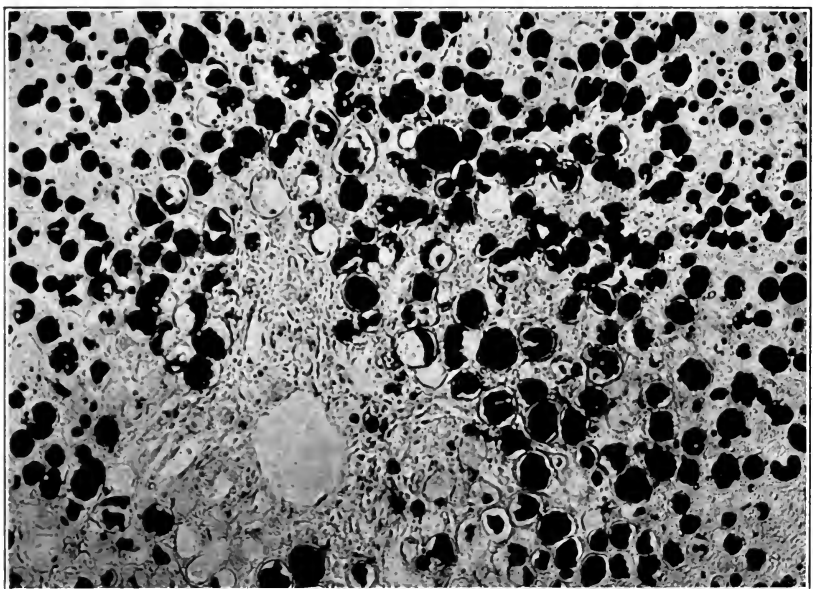


FIG. 2.—MAGNIFICATION 175 DIAMETERS.

LIVER SECTIONS OF ORGANIC-PHOSPHORUS-FED RABBIT NO. 2.  
[Fat stained black with osmic acid.]





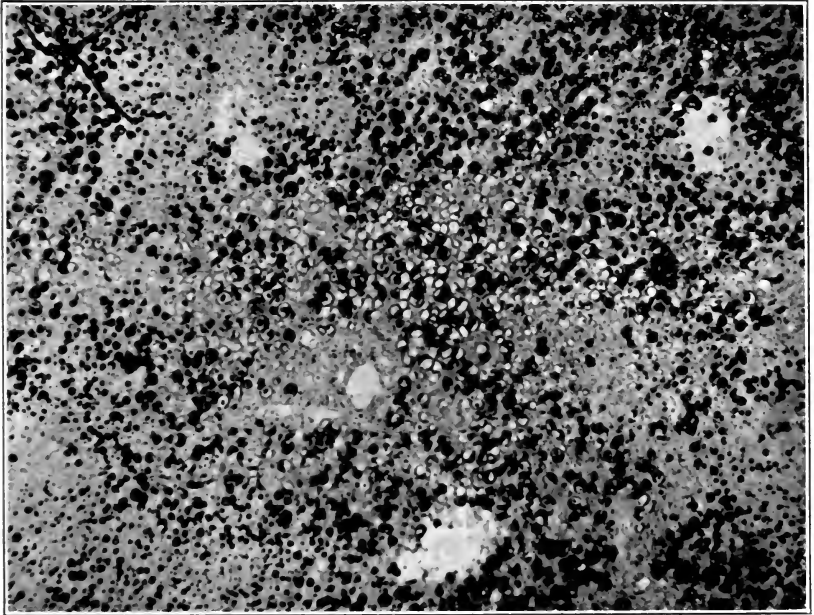


FIG. 1.—MAGNIFICATION 60 DIAMETERS.

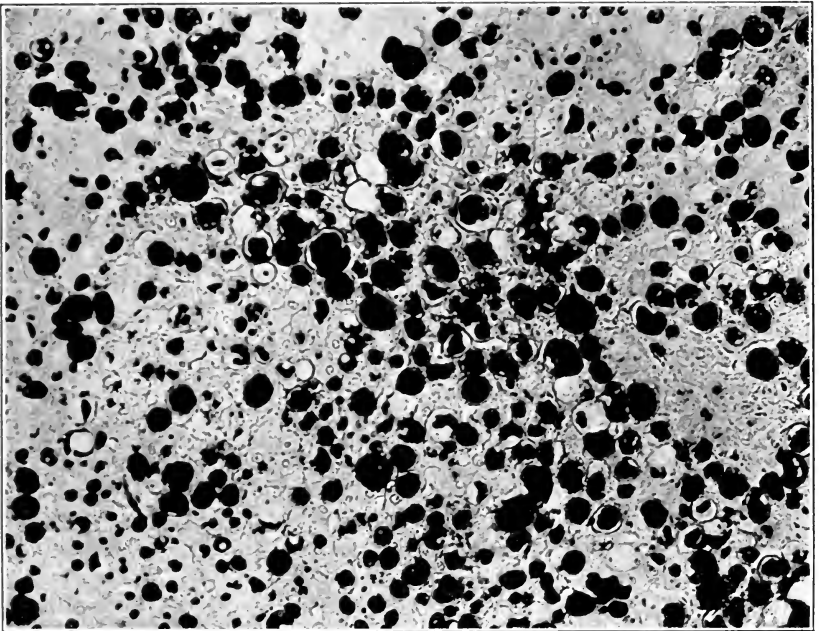


FIG. 2.—MAGNIFICATION 175 DIAMETERS.

LIVER SECTIONS OF INORGANIC-PHOSPHORUS-FED RABBIT NO. 4.  
[Fat stained black with osmic acid.]



a smaller proportion of the absorbed nitrogen than did the rabbits fed inorganic phosphorus. With a few exceptions, the daily balances were positive.

The rabbits fed organic phosphorus excreted less phosphorus through the kidneys than did those fed inorganic phosphorus, but they retained a larger proportion of the absorbed phosphorus.

#### PRINCIPAL FEEDING PERIOD.

The percentages of absorbed nitrogen retained in the case of the rabbits fed inorganic phosphorus show a wide variation, the average figure being practically the same as that for rabbit No. 1 fed organic phosphorus, rabbit No. 2 being excluded from the average. Accordingly, it is impossible to establish any effect produced on the nitrogen metabolism by the two forms of phosphorus fed.

A larger proportion of the ingested phosphorus was eliminated by the kidneys in the cases of the rabbits fed inorganic phosphorus than where the rabbits were fed organic phosphorus. The proportion of absorbed phosphorus retained is greater for the rabbits fed on organic phosphorus. The daily balances were positive with the exception of rabbit No. 2, which died two weeks before the close of the experiment.

The ether-alcohol-soluble phosphorus balances show that more phosphorus in this form was eliminated in the feces where phytin was fed than where inorganic phosphorus was fed.

No ether-alcohol-soluble phosphorus was found in the urine in any case, and it is doubtful if any organic phosphorus is present in the urine of a normal rabbit.

A smaller proportional amount of calcium was eliminated in the urine by the rabbits fed on inorganic phosphorus, but a larger proportional amount was retained in the body than in the cases of the rabbits fed on organic phosphorus. The same statement is true of magnesium.

#### POST-MORTEM EXAMINATION.

A large proportion of the phosphorus of the fresh intestines of rabbits is dissolved by water during the process of cleaning preparatory to analysis.

The bones of rabbits fed on phosphorus for several months, either organic or inorganic, show a higher content of ether-soluble matter than do the bones of normal rabbits, and they form a larger percentage of the body weight than in the case of normal rabbits.

The livers of the rabbits fed on organic phosphorus for several months show fatty degeneration as well as fatty infiltration. Of the livers of the inorganic phosphorus fed rabbits No. 4 shows both fatty degeneration and fatty infiltration; in the case of No. 3, only slight fatty infiltration is shown. The livers are enlarged and contain

considerably more nitrogen and phosphoric acid than normal livers when calculated to a water and fat-free basis. In these experiments the degeneration and infiltration were most marked when organic phosphorus was ingested.

The brains and nerves of the rabbits fed on organic phosphorus yield a larger percentage of ether-alcohol soluble phosphorus than those of normal rabbits, while the brains and nerves of those fed inorganic phosphorus show lower figures. There is also a larger percentage content of ether-soluble material in the brains and nerves of the phosphorus-fed rabbits than is normal.

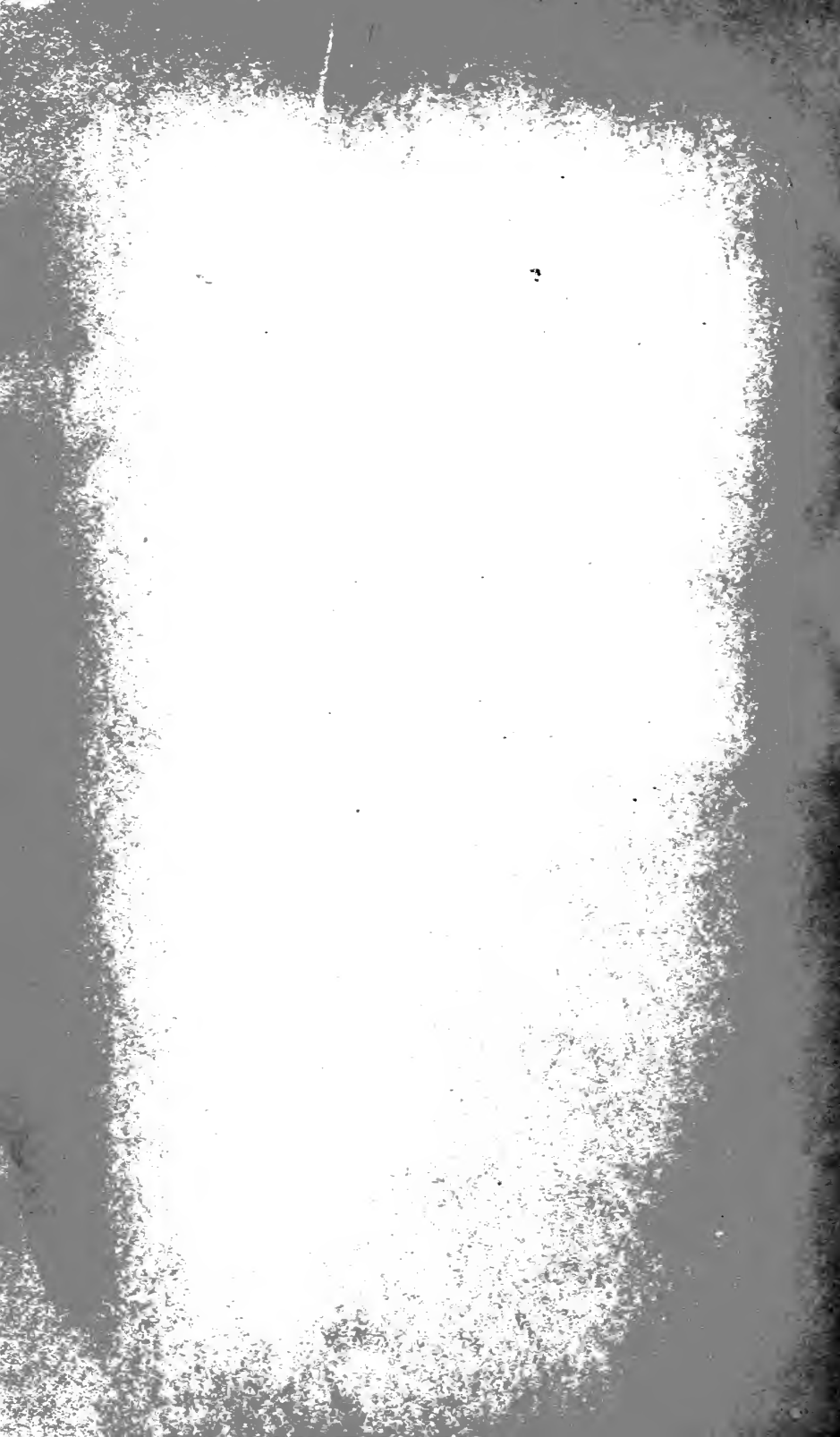
The tendency of organic phosphorus, especially in the form of phytin, to produce fat was strikingly indicated in this experiment as in the work of Jordan, Hart, and Patten. The autopsies showed that all the phosphorus-fed rabbits were in an abnormal condition, the livers being especially affected, and the histological examination confirmed the autopsy findings.

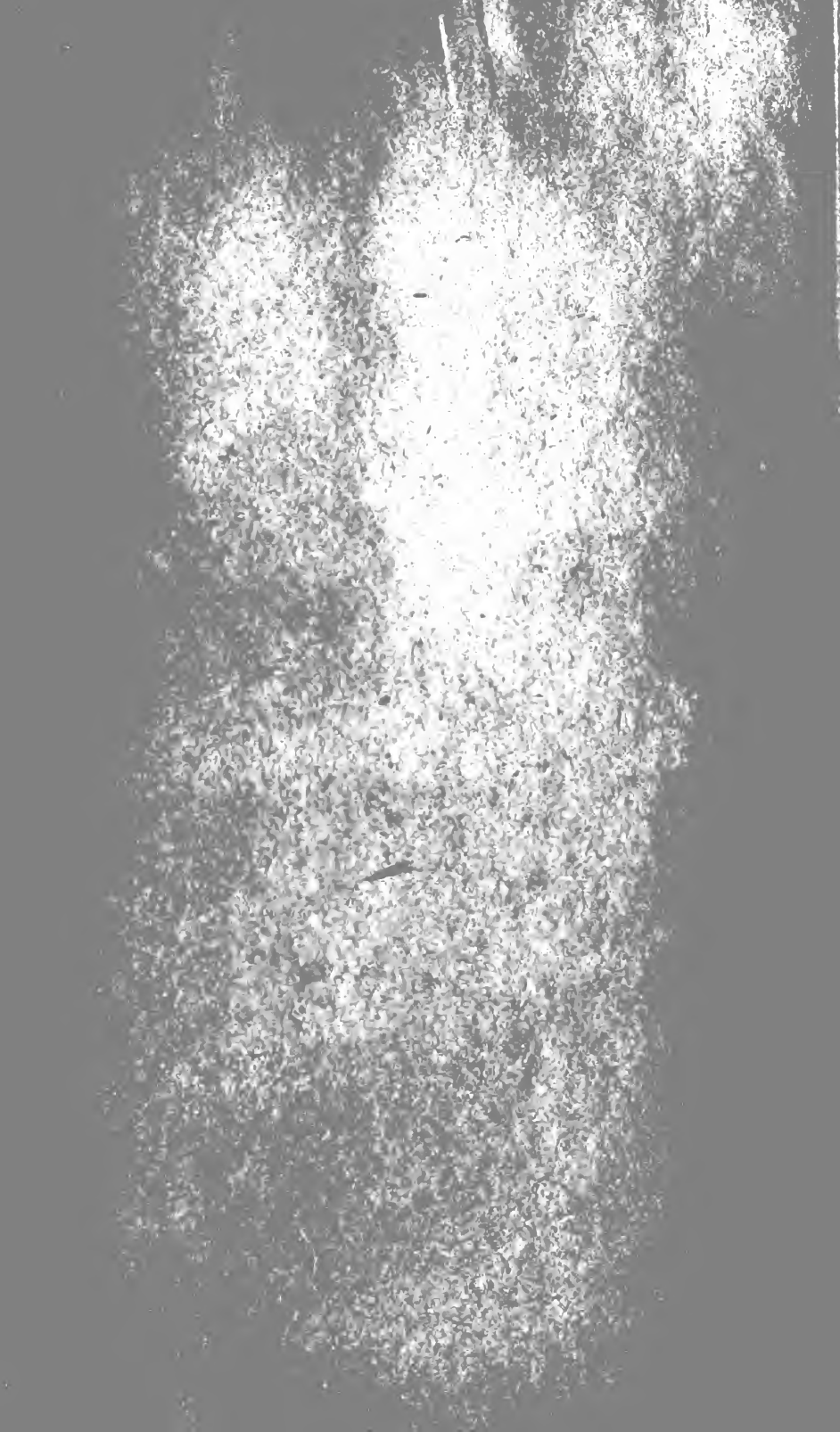


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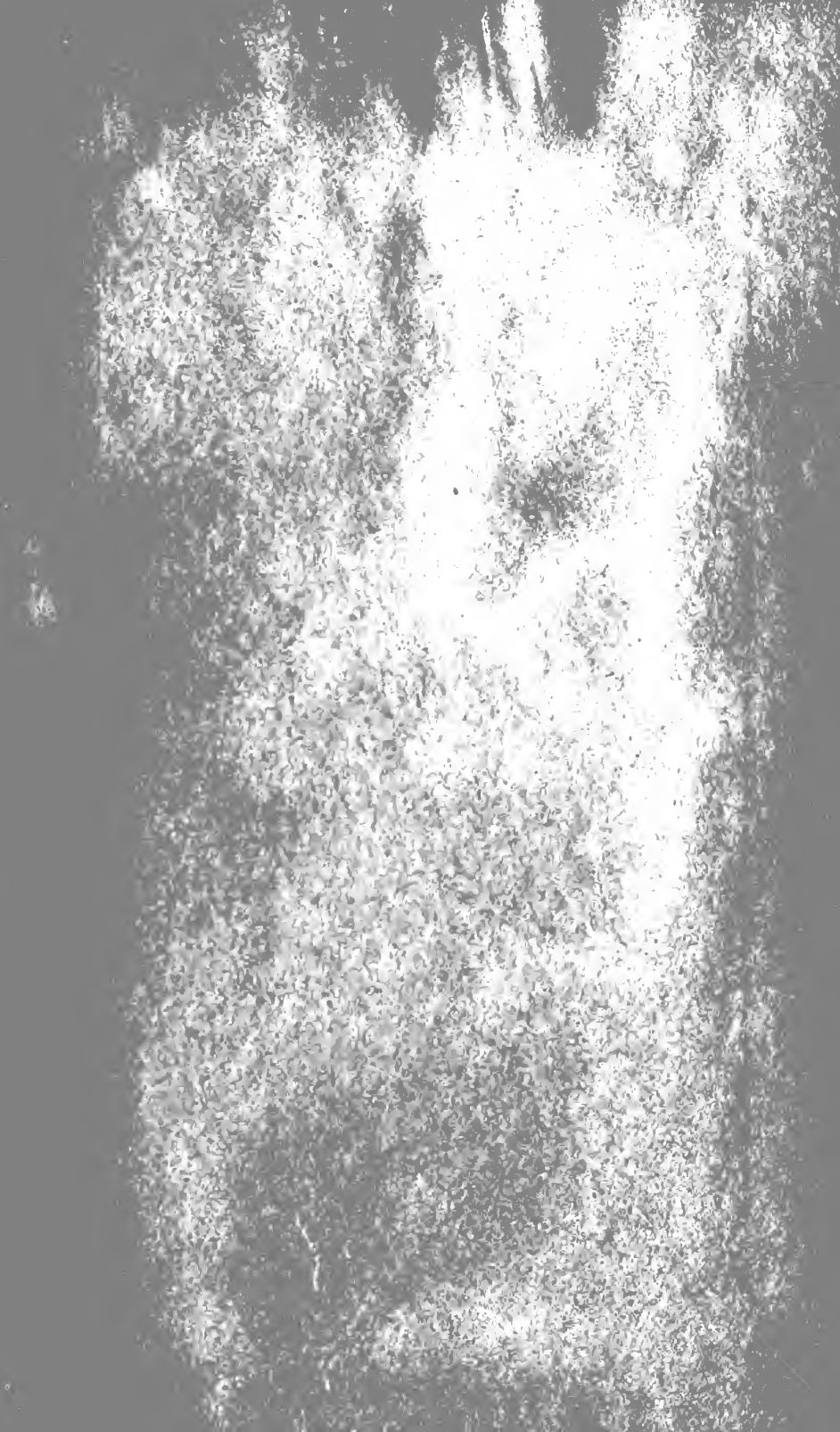












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