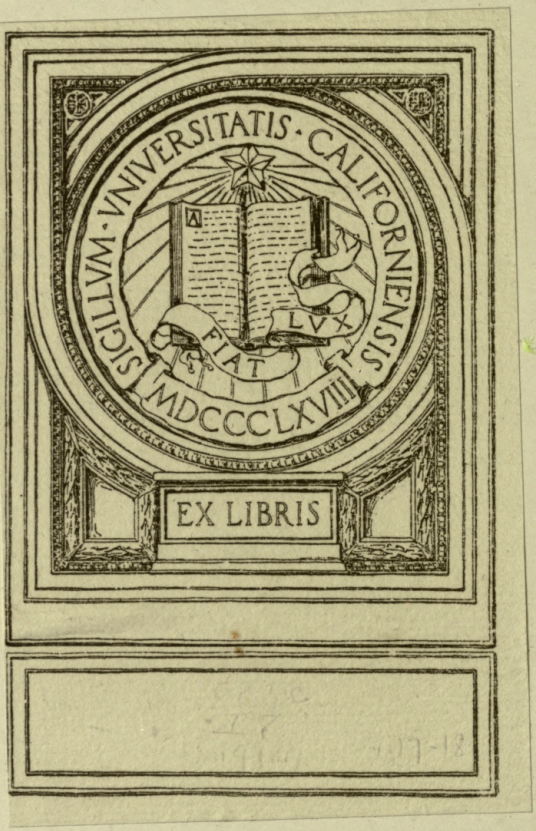

SIGILLVM · VNIVERSITATIS · CALIFORNIENSIS
LVX
FIAT
MDCCCLXVIII
EX LIBRIS

ORIGINAL COMMUNICATIONS

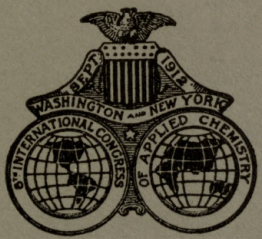
EIGHTH INTERNATIONAL

CONGRESS OF APPLIED CHEMISTRY

Washington and New York

September 4 to 13, 1912

SECTION VIIIb
PHARMACEUTICAL CHEMISTRY



VOL. XVII

ORIGINAL COMMUNICATIONS

EIGHTH INTERNATIONAL

CONGRESS
OF APPLIED CHEMISTRY

Washington and New York

September 4 to 13, 1912

SECTION VIIIb
PHARMACEUTICAL CHEMISTRY

VOL. XVII

The matter contained in this volume is printed in exact accordance with the manuscript submitted, as provided for in the rules governing papers and publications.

La matière de ce volume a été imprimée strictement d'accord avec le manuscrit fourni et les règles gouvernant tous les documents et publications.

Die in diesem Heft enthaltenen Beiträge sind genau in Übereinstimmung mit den uns unterbreiteten Manuskripten gedruckt, in Gemässheit der für Beiträge und Verlagsartikel geltenden Bestimmungen.

La materia di questo volume e stampata in accordo al manoscritto presentato ed in base alle regole que governano i documenti e le publicazioni.

THE RUMFORD PRESS
CONCORD·N·H·U·S·A·

ORIGINAL COMMUNICATIONS

TO THE

EIGHTH INTERNATIONAL CONGRESS

OF

APPLIED CHEMISTRY

APPROVED

BY THE

COMMITTEE ON PAPERS AND PUBLICATIONS

IRVING W. FAY, CHAIRMAN

T. LYNTON BRIGGS
F. W. FRERICHS
A. C. LANGMUIR
JOHN C. OLSEN
JOSEPH W. RICHARDS
E. F. ROEBER
A. F. SEEKER

SECTION VIII*b*.—PHARMACEUTICAL CHEMISTRY

Executive Committee

President: Joseph P. Remington, Ph.M.
Vice-President: V. Coblentz, Ph.D.
Secretary: George D. Rosengarten, Ph.D.
Albert Plaut
Samuel P. Sadtler, Ph.D.

Sectional Committee

T. B. Aldrich, Ph.D.
John F. Anderson, M.D.
Francis D. Dodge, Ph.D.
A. R. L. Dohme, Ph.D.
F. R. Eldred
Samuel W. Fairchild, M.S.
William H. Erhart
F. W. Frerichs, Ph.D.
Walter S. Haines, M.D.
E. M. Houghton, M.D.
Lyman F. Kebler, M.D.
J. A. Koch, Ph.D., Sc.D.
Henry Kraemer, Ph.D.
Charles H. LaWall, Ph.M.
John U. Lloyd, Phar.M., Ph.D., LL.D.
John H. Long, Sc.D.
A. B. Lyons, M.D.
E. Mallinckrodt, Jr.
B. L. Murray, Ph.C., M.A.
M. J. Rosenau
Lucius E. Sayre, Ph.M.
George L. Schaefer
Louis Schaeffer
W. Jay Schieffelin, Ph.D.
A. B. Stevens, Ph.D.
Jokichi Takamine, Dr. Ing., Phar.D.
C. E. Vanderkleed, Phar.D.
Theodore Weicker
William T. Wenzell, M.D.
W. Hull Wickham, A.M.
Harvey W. Wiley, M.D., Ph.D., LL.D.

and the Sectional Executive Committee

VOLUME 17

SECTION VIIIb.—PHARMACEUTICAL CHEMISTRY

CONTENTS

THE EFFECT OF CULTIVATION UPON THE ALKALOIDAL CONTENT OF A. BELLADONNA

By Francis H. Carr
Dartford, Kent, England

Observations regarding the percentage of alkaloid contained in *Atropa Belladonna* plants have been recorded by various experimenters from time to time during the past sixty years.

A review of the whole of these results shows conclusively that there is a wide variation in the amount of alkaloid which occurs in belladonna. It is, however, a more difficult task to trace the conditions which determine these variations; clearly there are several such conditions, notably those of soil, climate and weather.

The author has certain results to record which furnish further evidence on this question; but since variations of climate considerably affect the alkaloidal contents, caution must be exercised in drawing generalisations from a set of experiments made in one locality.

The experiments in question have been carried out during the past seven years on the Wellcome Materia Medica Farm — a plot of land situated at Darenth, on the chalky downs of Kent and sloping to the south-west. In the near vicinity wild belladonna was to be occasionally found before these experiments commenced; since then it has become more common owing to the dissemination of the seed by birds.

The soil is light, permeable and chalky. The following is an analysis of a representative sample of the dry soil after removing pebbles, etc., which represent 20 per cent of its weight:

$CaCO_3$	11.4 per cent.
K_2O	0.4 per cent.
Fe_2O_3 and Al_2O_3	6.2 per cent.
H_3PO_4	0.6 per cent.
Total loss on ignition	9.5 per cent.
Insoluble in acids	72.2 per cent.

In such a soil and position the belladonna plant grows very freely. Analyses have shown that the stem, leaves and root of both the wild and the cultivated plants grown there contain a high percentage of alkaloid.

Most observers have stated that the cultivated plant contains less alkaloid than that which grows wild.[1] This statement, which is no doubt true of plants transported to a soil unsuited to them, is not confirmed by the author's experience with a soil naturally suited to the plant; indeed, the addition of fertilisers to the soil in some cases appears slightly to increase the percentage of alkaloid, and on the whole the cultivated plant has been found to contain a little more alkaloid than that grown wild.

Although the addition of manures to this soil is no disadvantage and may be slightly beneficial, yet where large quantities of nitrogenous fertilisers are employed somewhat lower percentages of alkaloid are observed. This appears to be due to the larger growth which results from such manuring — the larger growth producing more weight of woody fibre.

The analytical method adopted does not vary in principle from that usually employed. Ammonia was used to set free the alkaloid and with the usual precautions to avoid emulsions, the latter was extracted with chloroform, and shaken into 2 per cent sulphuric acid, it was then again set free with ammonia and shaken out with chloroform. The chloroform residue — usually crystalline — was titrated, using cochineal as indicator.

In the first instance two distinct strains of wild plant were employed; later that which gave the best results was alone cultivated.

Representative portions of the stem and leaf were gathered while the plant was in flower, and rapidly and completely dried at 25°-35° C.

The percentage of alkaloid found in the leaves and stem of the dried wild plant was 0.49; in the leaves and stem of the cultivated plant, during the seven years 1906-1912, the average percentage was 0.56. As other investigators have usually recorded about 0.45 per cent in the wild plant, it may be assumed that the plant employed was satisfactory; it therefore follows that the effect of cultivation has been beneficial.

With a view to testing the effect of modifying the plant food, ground which had been slightly manured was divided into plots and these were treated with various fertilisers, added at periodic intervals of three to four weeks during the growing season, March-July. The effect of the more common fertilisers is shown by the following table:

Effect of Fertilisers upon A. Belladonna

FERTILISERS			PERCENTAGE OF ALKALOID IN DRY STEM AND LEAF				
Treatment	Time of application	Amount of each application per acre	1906 Third year's plants	1907 Fourth year's plants	1910 First year's plants	1911 Second year's plants	1912 Third year's plants
Main crop			0.54	0.34	0.61	0.59	0.68
Farmyard manure	March	50 loads	0.54	0.34	0.61	0.53	0.71
Nitrate	March, April, May and June	2-cwt.	0.52	0.23	0.54	0.46	0.64
Calcium Cyanamide	"	1-cwt.			0.69	0.49	0.75
Basic Slag	"	2-cwt.	0.61		0.65	0.56	0.84
Superphosphate	"	5-cwt.	0.46		0.81	0.49	0.76
Potash	"	5-cwt.	0.61	0.4	0.75	0.53	0.69

In considering these results it must be remembered that the soil is naturally suited to the plant and the percentage of alkaloid obtained without added fertilisers is already high. The low figures obtained in 1907 were probably due to the seasonal conditions.

It is clear that atmospheric conditions have a modifying influence. In the following table the percentages of alkaloid present in the dry stem and leaf of the main crop are set out in conjunction with the weather conditions:

Year	Percentage of alkaloid in stem and leaf	[1]Total hours sunshine May 1st to June 30th	[1]Rainfall (Same period)
1905	0.38	387 hours	5.48 inches.
1906	0.54	361 hours	3.86 inches.
1907	0.34	290 hours	3.54 inches.
1909	0.48	387 hours	5.44 inches.
1910	0.61	360 hours	4.08 inches.
1911	0.59	404 hours	3.62 inches.
1912	0.68	Unusually dry and sunny season.	

It will be seen that the highest percentages of alkaloid were observed in the most sunny and driest seasons, while the low percentages found in 1905 and 1907 are explained by the heavy rainfall in the former and the lack of sunshine in the latter season.

As regards the question of the stage of growth at which the plant may best be collected results indicate that, while the amount of alkaloid may vary during the season from other causes, there is no marked variation due to different stages of growth from June to September except when the plant begins to fade, when there is a rapid loss of alkaloid.

Thus, stem and leaf gathered in different months of the same year have yielded the following percentages of alkaloid, which do not show any consistent variation with the calendar, but vary according to the weather:

Year	Month	Percentage of alkaloid	Character of weather during interval between taking samples
1905	August	0.38	Very wet.
1905	September	0.35	
1906	July	0.54	Very fine and very dry.
1906	October	0.64	
1907	June	0.33	Dull.
1907	August	0.34	
1909	June	0.41	Fine and dry.
1909	September	0.48	

[1]These figures were recorded in London, 16 miles distant, and acknowledgments are due to the Director of the British Meteorological Office who has courteously supplied them.

Slightly faded shoots having yellow foliage and taken from a healthy plant contained 0.21 per cent, while healthy shoots from the same plant contained 0.38 per cent.

The time of collecting the herb and leaf for use in pharmacy does not, therefore, appear to be a matter of importance except in relation to weather conditions, and so long as great care is exercised to avoid those parts of the plant in which the least withering or fading has commenced.

This precaution as applied to all drug yielding plants is well recognised by collectors, and the present instance confirms its value. The fact that the stem and leaves rapidly lose alkaloid when fading raises a question as to the fate of the alkaloid so lost. On this account, interest attaches to an experiment made with a view to seeing whether any of the alkaloid finds its way into the soil. A plant was grown in Pfeffer's culture solution and the solution tested from time to time for alkaloid, but no evidence whatever was obtained that any alkaloid was present in it at any stage—either before or after fading had commenced. Analysis of the healthy stem and leaf showed it to contain a normal amount of alkaloid, namely, 0.56 per cent. It follows from this experiment that during fading the alkaloid is either decomposed or passes back into the root.

Experiments have been made from year to year to test the effect of growing belladonna in partial shade. Shades were constructed of:

1. Wooden lathes—designed to shut off half the direct sunlight.
2. White muslin.
3. Red muslin.
4. Green muslin.

The resultant percentages are tabulated below:

		Shaded By			
Year	In open	Lath shade	White muslin	Red muslin	Green muslin
1906	0.54	0.39			
1907	0.34	0.40	0.60	0.63	0.75
1910	0.61		0.57	0.8	1.08
1911	0.59		0.47	0.6	0.49

The lath shade in the sunny year 1906 reduced the amount of alkaloid, and in the dull season of 1907 it appears to have had no effect, though in fact the figures show an insignificant increase. The coloured muslin shades made the growth somewhat attenuated. The green shade had the effect of reducing the amount of inflorescence and at the same time increasing the amount of alkaloid. In 1911 an experiment was therefore made to see whether the removal of all flower buds would raise the percentage of alkaloid of a plant grown in the open. The result was negative:

Representative plant	0.59 per cent.
Suppressed inflorescence	0.54 per cent.

Belladonna Roots

Belladonna root of commerce varies greatly in alkaloidal strength. In a number of analyses made of commercial roots, variations from 0.27 to 0.69 per cent have occurred. The average of twenty-one analyses of German and Austrian commercial roots was 0.40 per cent.

Other observers have recorded similar results.

Chevalier (Compt. Rend. 1910, *150*, 344) gives the following figures for continental roots:

French	0.300—0.450 per cent.
Austrian	0.251—0.372 per cent.
Italian	0.107—0.187 per cent.

Henderson (Pharm. J., 1905, *75*, 191) has shown the average of thirty samples of foreign root to be 0.3 per cent.

It is interesting to observe that the average of nine samples of root grown at Darenth is 0.54 per cent.

In order to determine whether this variation was due to collecting at different times of the year, roots from the same plot, derived from second year's plants which had been sown at the same time, were dug up at intervals and dried. The following is a record of the analysis of these samples:

March 1911	0.56 per cent.
May 1911	0.59 per cent.
June 1911	0.53 per cent.
August 1911	0.50 per cent.
December 1911	0.59 per cent.

The amount of variation throughout the year is thus seen to be very small; there appears, however, to be slightly less alkaloid present during August, when the fruit is ripening. We must therefore seek other explanations for the low percentage of alkaloid present in commercial belladonna root. If one considers the figures recorded by various observers it appears that in the warmest climates, such as those of Italy and Austria, the lowest proportion of alkaloid is to be observed; and this would account for the high reputation of English belladonna. But no such generalisation can be regarded as established until plants grown in those countries in a suitable soil and under careful observation have been submitted to analysis. That such an explanation is not improbable is clear from the published facts relating to other alkaloid-producing plants, which show that different amounts of alkaloid are formed in different latitudes. For instance, Dunstan (Bull. Imp. Inst. 1905, 222) has recorded that *Hyoscyamus muticus* grown in Egypt produces 0.6 to 1.2 per cent of Hyoscyamine, while the same species grown in India produces only 0.3 to 0.4 per cent. On the other hand, if the harvesting of the crops is done with less care in one country than in another, more decomposition may take place during the process of drying and so cause the observed differences.

In whatever latitude belladonna is grown, it will doubtless be found that the composition of the soil, the use of fertilisers, and seasonal conditions make for small variations.

THE ASSAY OF BENZALDEHYDE, AND OIL OF BITTER ALMOND

By Francis D. Dodge
Bayonne, N. J.

The accurate determination of benzaldehyde is a matter of some importance, inasmuch as this compound as well as the natural oil of bitter almond, are official in the U. S. Pharmacopœia.

Of the processes available, I have had occasion to study the following:

1. The U. S. P. process (Sadtler).
2. The Iodometric (Ripper).
3. The Hydrazone process (Denner).
4. The Oxime process (Walther, Bennett).
5. A method based on Cannizzaro's reaction.

In the U. S. P. a method has been adopted for the estimation of both benzaldehyde and citral, based on the reactions of these aldehydes with a solution of sodium sulphite.

The conditions in the two cases are, however, not strictly analogous. In 1890 (Am. Ch. J. 12.553), I observed the peculiar behavior of citral with bisulphite of soda, and described a method for the isolation of this aldehyde, which has since come into general use.

Tiemann (Ber. 1898, 3317) further studied this reaction and formulated it as follows:

$$C_9H_{15}-COH + 2Na_2SO_3 + 2H_2O = C_9H_{17}(SO_3Na)_2COH + 2NaOH.$$

The aldehyde being doubly unsaturated can take up two molecules of $NaHSO_3$, with liberation of a corresponding amount of NaOH. But the reaction, being reversible, ceases at a certain concentration of NaOH. If the latter be neutralized, however, as fast as it is formed, the reaction proceeds smoothly, with quantitative formation of the di-sulphonate.

Theoretically, then, the estimation of the citral by titration of the free alkali liberated, appears unobjectionable. But in the case of benzaldehyde, there is no possible formation of a stable sulphonate. The reaction:

$C_6H_5-COH + Na_2SO_3 + H_2O = C_6H_5COH.$ $NaHSO_3 + NaOH$ leads to the well-known "bisulphite-compound" of benzaldehyde, whose structure is even yet uncertain, being variously formulated as oxy-sulphonate, oxy-sulphurous ester-salt, or "oxonium compound."

However that may be, the compound is certainly less adapted to accurate titration than the citral derivative, for it is largely dissociated in aqueous solution, especially when heated, and, in my experience, the occasional satisfactory results appear rather to be due to compensating errors.

Furthermore, it is well known that the titration of the sulphite solution is an uncertain matter, and, in the case of benzaldehyde, the prescribed conditions favor the oxidation of the aldehyde before it can be gotten into solution.

In fact, consistent results could not be obtained by this method. Owing to the high percentage involved, the experimental error is a serious difficulty. In the case of oil of lemon, it is not of great moment whether the amount of aldehyde is found to be 4.0% or 4.2%, but the same relative error in the case of nearly pure benzaldehyde amounts to 4.%.

The method of Ripper (Monatsch. f. Ch. 21. 1079) gave promise of better results, inasmuch as the aldehyde can be quickly dissolved, and all heating is avoided, thus reducing to a minimum any oxidation and dissociation.

The process is based on the fact that the "bisulphite compound" is not immediately oxidized by iodine in the cold.

The aldehyde is dissolved in standard $NaHSO_3$ solution, and when the reaction is complete, the excess of $NaHSO_3$ is titrated with standard iodine.

As was to be expected, the end point is not permanent, owing to a slow dissociation of the bisulphite compound, which increases with the temperature.

The presence of alcohol also appears to interfere.

By keeping the temperature low, and allowing sufficient time for a complete reaction between the aldehyde and the bisulphite, fairly concordant results, of an accuracy sufficient for technical work, could be obtained.

But, on the whole, it appears unlikely that any method based on the normal bisulphite reaction of aldehydes in dilute solutions can be made to yield better than approximate values.[1]

We obtained the best results under the following conditions: 0.15 gm. aldehyde is weighed into a flask containing exactly 25 cc. of about $\frac{N}{5}$ sodium bisulphite, and dissolved by gentle shaking. The flask is corked and let stand 1½ to 2 hours in ice-water. It is then titrated, ice-cold with $\frac{N}{10}$ iodine, and starch indicator. A blank test of 25 cc. of the bisulphite solution is treated in all respects similarly. The amount of iodine used in the assay is subtracted from that used in the blank, and the difference calculated to benzaldehyde (1 cc. $\frac{N}{10}$ iodine = .0053 benzaldehyde).

The end point is assumed to be when the blue color is permanent for a few seconds.

ANALYSES

Sample 1. Artificial. Free from chlorine. S. G. 1.0435 $\frac{25°}{25°}$ (contained traces of water).

Found: 96.6, 97.6.

Sample 2. (do) S. G. 1.0453.

Found: 94.8, 94.9, 95.2, 95.9, 96.2, 96.4, 97.

Sample 3. Natural, free from HCN.

Found: 96.4, 97.4.

Sample 4. Artificial S. G. 1.042.

Found: 99.0%.

We have tried, also, the oxime method as employed for oil of lemon (Walther, Pharm. Centr. 1899. 40. 621) (Bennett, Analyst, 1909. 14).

Sample 1. 93.9%.

Sample 2. 93.5, 95, 95.1, 94.1, 93.6.

The process appears to offer no advantage over the iodometric one.

[1]The dissociation of various bisulphite compounds has been studied by Kerp (Arbeiten a.d. Kaiserl. Ges. Amt. (1904) 180; (1907) 231, 269.

The hydrazone method of Denner (Z. anal. ch. 29. 228) and Denis & Dunbar (J. Ind. Ch. I. 256) was found quite satisfactory, but the instability of the reagent is a disadvantage where assays are infrequent.

0.1 to 0.15 gm. aldehyde is dissolved in 10 cc. 50% alcohol, and treated with 10 cc. phenyl hydrazine-acetate solution. Further manipulation as recommended by Denis & Dunbar.

Sample 1. Artificial (found 100.7, 100.9%).

Sample 3. Natural (found 95.1, 95.2%).

Sample 5. Artificial. (found 99.1%).

Numerous other assays by this method have shown that the results are generally a little high.

METHOD UTILIZING CANNIZZARO'S REACTION

The reaction of benzaldehyde with strong alkali is as follows:

$$2C_6H_5COH + KOH = C_6H_5COOK + C_6H_5 - CH_2OH.$$

A series of experiments were made to determine the availability of this process for analytical purposes. It was found that, with alcoholic potassium hydrate weaker than 2N, the reaction was incomplete, and uncertain, but with 2.5N solution good results were possible.

More uniform values also were obtained when the mixture was allowed to stand at room temperature 24 hours than by heating for a shorter time.

ANALYSES: PURE ARTIFICIAL BENZALDEHYDE

1. 1.0141 gm. + 10 cc. 2.5 alcoholic KOH.

Stood 24 hours.

Blank test, 10 cc. 2.5N KOH required 50.8 cc. $\frac{n}{2}$ HCl.

Assay, required 41.2 cc. HCl.

used, 9.6 cc. HCl.

$$\frac{9.6 \times 106}{1.0141} = 100.3\%.$$

2. 1.0489 gm. required 9.8 cc. $\frac{n}{2}$ KOH.

99.03%.

3. 2.0641 gm. required 19.3 cc. $\frac{n}{2}$ KOH

99.1.

Great care, of course, is necessary in handling such a strong solution. An error of 0.05 cc. amounts to from 1 to 2.5% of benzaldehyde.

The method, however, is simple, and for pharmacopœal testing would appear worthy of consideration.

Assays of Oil Bitter Almond in this manner were not very satisfactory. The saponification of the nitril, to mandelic acid, appeared to be incomplete, and the end reaction with phenolphthalein was uncertain, probably owing to the presence of ammonia.

In applying any of the assay methods for benzaldehyde to the oil of bitter almond, the effect of the presence of hydrocyanic acid must be kept in mind.

It is well known that the hydrocyanic acid exists in combination with the aldehyde as "cyanhydrin", or mandelic nitril, $C_6H_5-CH(OH).CN$.

It has also been shown (Müller, Bev. 4.980, Bucherer and Grolée, Bev. 39.1224) that the bisulphite compounds of aldehydes are decomposed by hydrocyanic acid with formation of the corresponding nitrils. Hence those methods which depend on the bisulphite reaction will estimate only the free aldehyde. Wirth (Arch. der Pharm. 249. 382.) has also recently shown that, in the cold, only the free aldehyde is precipitated as hydrazone. With the oxime method, also the presence of hydrocyanic seems to cause irregularities in the titration.

ANALYSES

6. One Bitter Almond, Pure. S. G. 1.057 $\frac{25°}{25°}$ HCN (U. S. P. Assay) 3.58, 3.59%.

This amount of HCN is equivalent to 17.6% nitril, leaving for maximum free aldehyde, 82.4% (calc.).

Found: Free aldehyde:

82.1, 82, 81.2, 81.3, (Iodometric).
78.9, (oxime).
83.2, 83.1, (Hydrazone).

7. (do) S. G. 1.0536 $\frac{25°}{25°}$

HCN. 2.8% (calc. free aldehyde—86.2%).

Found: 81.4, 80. (Iodometric).
83.6, 87.2 (Hydrazone).

8. (do) S. G. 1.0513 $\frac{25°}{25°}$

HCN—2.4% (calc. free aldehyde—88.2).

Found: 85.2 (Hydrazone).

It will be noticed that the U. S. P. limits of 4% HCN and 85% benzaldehyde are incompatible, the range of 2–4% HCN corresponding to a free aldehyde content of 80.3 to 90.1%.

OTHER CONSTITUENTS OF ALMOND OIL

The odor and flavor of the natural benzaldehyde from almond oil have been generally considered superior to those of the best artificial product, and analyses of the former have at times indicated the possibility of the presence of some other constituent.

For further information on this point I proceeded as follows:

60 gms. of oil, freed from hydrocyanic acid, by distillation with lime and ferrous sulphate, (S. G. 1.0435 $\frac{25°}{25°}$) was dissolved in 100 cc. ether, and precipitated by 200 gms. concentrated sodium bisulphite solution.

After 18 hours the crystals were filtered off, and washed with ether. The ether extract, after washing with a little sodium carbonate solution, was evaporated in a vacuum desiccator. The residue was a yellow oil, heavier than water, with an agreeable, characteristic odor, and weighed 0.1 gm. or approximately 0.2%.

It has not yet been identified.

Although small in amount, it is probably not without influence on the aroma of the natural oil.

Artificial benzaldehyde under the same treatment yielded traces of oil, without marked odor.

Abstract

THE ASSAY PROCESSES OF CINCHONA

By A. R. L. Dohme and H. Engelhardt
Baltimore, Maryland

The various assay processes recommended for assaying cinchona bark are given. The methods are in chronological order arranged according to the menstruum employed for extracting the bark and the alkali used for liberating the alkaloids. Various methods recommended for separating the four most important cinchona alkaloids are mentioned also. The paper, which does not present any new facts, was compiled in order to facilitate further research on the assay of cinchona, because by most of the methods, especially those proposed for estimating quinine, absolutely accurate results are not obtained.

1. Amer. Jour. of Pharmacy, 1875, page 3.
2. Zeitschr. der Chemie 2. Ser. II, page 435.
3. Zeitschrift des Oester. Apoth. Ver. VI, page 351.
4. Pharmaceut. Centralh. 1871, No. 2.
5. Nieuw. Tijdsch. voor d. Pharm. in Nederl. 1869, page 322, and 1870, page 7.
6. N. Jahrbuch fuer Pharmacie XXXIII, page 1.
7. Nieuw. Tijdsch. voor d. Pharm. in Ned. 1871, page 143.
8. Nieuw. Tijdsch. voor d. Pharm. in Ned. 1875, page 161.
9. Arch. der Pharm. 1882, pages 722 and 812.
10. Proceedings A. Ph. A. 1884, page 474.
11. Pharm. Journ. and Trans. Ser. III, Vol. VI, page 361.
12. Journ. de Pharm. et Chim. 4. Ser. XII, page 81.
13. L'Union Pharmaceutique, 1899.
14. Pharmazeutische Zeitung, 1881, page 224.
15. Nieuw. Tijdsch. voor d. Pharm. in Ned. 1878, No. 4 and 5, page 152.
16. Journ. de Pharm. et Chim. IV, Vol. 29, pages 135-139.
17. Ephemeris 1881.
18. Trans. Brit. Pharm. Confer. 1905, page 471.
19. Pharm. Journ and Trans. 1905, page 579.
20. Apotheker Zeitung, 1896, page 147.
21. Zeitschrift fuer angew. Chem. 1901, page 313.
22. Journ. de Pharm. et Chem. 1889, page 523.
23. Journ. de Pharm. et Chem. 1904, page 479.
24. Bull. Sciences Pharmacol. 1906, page 365.
25. Apotheker Zeitung, 1899, No. 76.
26. Schweiz. Wochensch. fuer Chem. und Pharm. 1903, page 265.

27. Jahresbericht. 1905, Wien-Klosterneuburg.
28. Berichte der Deutschen Pharm. Gesell. 1910, page 316.
29. Nieuw. Tijdsch. voor d. Pharm. in Ned. 1870, page 146.
30. Pharm. Zentralhalle X, pages 137 and 145.
31. Bull. de l'Acad. Roy. de Belge, 1902, through Chemiker, Zeitg. Rep. 1902, page 117.
32. l. c. 23.
33. Chemist and Druggist, 1875, page 218.
34. Archiv. der Pharm. (3) XIX, page 85.
35. Chemiker Zeitung, 1881, No. 50.
36. Nouv. Remèdes, 1882, page 258.
37. Archiv. der Pharm. (3) XIX, page 85.
38. Pharmaceutische Zeitsch. fuer Russland, 1882, page 249.
39. Schweizer. Wochensch. fuer Chem. und Pharm. XX, page 9.
40. Journ. de Pharm. et Chim. 5, VIII, page 481.
41. Archiv. der Pharm. 1888, page 382.
42. Schweiz. Wochensch. fuer Chemie und Pharm. 1891, page 147.
43. Pharm. Zentralhalle 1892, page 594.
44. Pharm. Weekbl. 1903, No. 2.
45. Pharm. Rundschau, 1894, page 57.
46. Festschrift der 50 jaehrigen Stiftungsfeier des Schweiz. Apoth. Verein, through Pharm. Zeitung, 1893, page 786.
47. Schweiz. Wochenschrift fuer Chemie und Pharm. 1895, page 449.
48. Journ. de Pharm. et Chim. 1904, page 479.
49. Schweiz. Wochensch. fuer Chemie und Pharm. 1903, page 523.
50. Caesar et Loretz, Geschaeftsberichte, 1904.
51. Pharm. Zeitschr. fuer Russland, 1863, No. 24.
52. Pharm. Zentralhalle XII, pages 201 and 209.
53. Archiv. der Pharm. CXCVII, page 97.
54. Upsala Laekarefoerenings Foerhandlingar VI, page 268.
55. Inaugural Dissert. Dorpat, 1889.
56. Nieuw. Tijdsch. voor de Pharm. in Ned., 1869, page 289.
57. Archiv. der Pharm. CXCVII, page 99.
58. Nieuw. Tijdsch. voor d. Pharm. in Ned. 1880, pages 16-22.
59. Americ. Journ. of Pharm. 1882, page 548.
60. Yearbook of Pharmacy, 1882, page 488.
61. American Journ. of Pharm. 1885, page 622.
62. Caesar und Loretz, Geschaeftsberichte, 1903.
63. Caesar und Loretz, Geschaeftsberichte, 1905.
64. Jahresbericht, Wien-Klosterneuburg, 1906.
65. Caesar und Loretz, Geschaeftsberichte, 1907.
66. Pharmaceutische Post. 1906, page 345.
67. Schweiz. Wochensch. fuer Chem. und Pharm. 1886, No. 24.
68. Archiv. der Pharm. CXCVII, page 99.
69. Ephemeris of Materia Medica through Pharm. Zeitg, 1899.
70. L'Orosi V, page 1.
71. Apotheker Zeitung, 1898, page 668.
72. Pharm. Journ. and Trans. 2 Ser. XI, page 222.
73. Yearbook of Pharmacy, 1878, page 550.
74. Apotheker Zeitung, 1907, page 748.
75. Pharm. Journ. and Trans. Ser. III, Vol. VI, page 461.
76. Journ. de Pharm. et Chim. XXI, No. 4.

A STUDY OF AMERICAN GROWN CANNABIS IN COMPARISON WITH SAMPLES FROM VARIOUS OTHER SOURCES

By C. R. Eckler and F. A. Miller
Indianapolis, Indiana

Several factors have recently given rise to considerable comment on the subject of American Cannabis. Of these, perhaps the most important are: the increased cost of the Indian drug, resulting in the search for a cheaper product; the none too well supported claims made by some investigators, leading, we believe, to a false conception of the activity of commercial lots of drug; the question as to whether or not an active variety can be successfully cultivated in this country on a commercial scale; and the question of the feasibility of including the American variety in the coming revision of the United States Pharmacopœia. The influence upon the activity of the drug of such factors as soil, climate, geographical location, time of harvesting, method of curing, and parts of plant included, are also of interest.

Famulener and Lyons[1] claim that the character of the soil and the geographical area where grown, primarily influence the activity of the plant. Holmes[2] reported on French, African and Indian Cannabis in 1905 to the effect that although the French and African varieties indicated some activity, they were not nearly so active as the Indian drug and could not be recommended for manufacturing purposes. He reported the African as being a little more active than the French. Houghton and Hamilton,[3] in 1908, named seven different sources from which they had tested samples, three being within the United States. In their conclusions they claim that Cannabis sativa, when grown in various localities of the United States, is found to be fully as active as the best imported Indian grown Cannabis. True and Klugh[4] grew plants

[1]Famulener & Lyons: Proc. Am. Pharm. Assoc., 1903, 51, 240.
[2]Holmes: Pharm. Jr., 1905, 74, 550.
[3]Houghton & Hamilton: Am. Jr. Pharm., 1908, 80, 21.
[4]True & Klugh: Proc. Am. Pharm. Assoc., 1909, 57, 843.

from foreign seed and commercial drug, at Washington and southern Texas. They reported that the home grown drug was found to be fully equal in efficiency to the imported article. Hamilton[1] called attention to the fact that "various investigators," whom he failed to name, have examined American hemp and obtained results which indicated that the influence of soil and climate does not effect the quality of the extract.

Our interest in the different phases of the Cannabis situation has led us to report our experience with various commercial samples of the drug, and to state in brief the results of our work on the experimental cultivation of the American and Indian varieties. Materials for these investigations were obtained by growing plots, experimentally, both from the foreign and native seed. The following tests were carried out on various samples collected from these plots.

CULTIVATED CANNABIS

Where grown	No. of Sample	Percentage activity compared with good Indian grown samples
Plot A	B-176	Approximately 40%
Plot A	1494	Approximately 50%
Plot B	B-596	Approximately 60%
Plot C	B-770	Not more than 50%
Plot C	B-771	Not more than 50%
	B-703	Approximately 65%
Plot D	B-177	Approximately 40%
Plot E	1493	Approximately 40%
	B-437	Approximately 50%
	B-693	Not more than 50%

The samples, on which the foregoing assays were made, were obtained under the following conditions: Seeds from an active commercial lot of Indian Cannabis were taken for experimental planting. A good stand of plants was easily obtained from these seeds. The plants were grown under ordinary agricultural conditions, upon a soil consisting of rather poor clay loam. The plants as observed on a plot (*A*) thirty by sixty feet, exhibited a

[1]Hamilton: Jr. Am. Pharm. Assoc., 1912, 1, 201.

wide range of variations. These variations were indicated by such botanical characteristics as size, color, and form, as found in leaf, plant, and inflorescence, date of flowering and time of maturity. Twelve plants from this plot (*A*) were of dwarf habit, from one to two feet high, and free from branches. These plants flowered early. They produced pistillate flower clusters which were heavy, compact, and leafless, averaging from two to four inches in length. They bore much resin and possessed the characteristic odor of Indian Cannabis. The three earliest flowering of these were selected for seed plants. August 17th, 1909, the pistillate inflorescence of the twelve dwarfs was collected for testing. The sample was cured at room temperature and designated by No. B-176. The remainder of the plants in plot *A* were from three to seven feet high, much branched, and produced small inconspicuous flower clusters which were late in appearing. They showed but few resin bearing glands and did not possess the odor of foreign Cannabis to such a marked degree as noticed in the dwarf plants. August 24th, 1909, the pistillate tops, including several inches of leafy stem, were collected for testing. The sample thus obtained was dried at room temperature, and designated by No. 1494.

The seeds selected from the three early flowering dwarfs were planted the following year on plot B. The plants obtained were a great improvement over the parents of the previous year. They averaged from two to five feet in height and were more branched. The inflorescence was long (averaging over twelve inches), dense, heavy, and carried much resin. Individual flower clusters were observed, measuring fourteen inches in length and bearing no leaves except the small bracts subtending the flower clusters. September 7th, 1910, a collection was made from twelve of the best plants. The sample thus obtained was cured at room temperature, and tested under No. B-596.

At the same time, seed plants were selected. These selections were made for the purpose of continuing and improving the strain, as follows: first, from one plant bearing the largest inflorescence, which in this instance measured fourteen inches in length; second, from one plant showing a deep purple color in all parts; and third, from twelve of the best remaining plants, the size of the inflorescence serving as a basis for the selection.

The following year the seeds from these selections were planted on plot C. The resulting crop consisted of uniform plants, very much like the parents in most cases. One marked exception was the appearance of the purple color in many plants from each of the other selections. It predominated, however, in the second selection. The first selection resulted in low, almost unbranched individuals. The pistillate flowering tops averaged ten inches in length, and produced more leaves than the parent form. A collection was made from these plants, for testing, on October 13th, 1911. This sample was taken from the twelve best plants and tested under No. B-770. The second selection gave rise to individuals, all but a small proportion of which possessed the purple characteristic to a marked degree. The pistillate tops averaged one foot in length but were of a more interrupted nature and not so dense as noted in the parent plants. A collection was made from these plants October 13th, 1911, and designated by No. B-771. No collection was made from the third selection. From sample B-770 three individual plant selections have again been made; first from one plant four feet high, and unbranched, which produced a large, single leafless inflorescence; second, from one plant three feet high, unbranched, which produced a large, single leafy inflorescence; and the third from one plant four feet high, which was divided near the top into several short branches. All of these produced good flower clusters, with numerous leaves. The strain showing the purple character was discontinued.

Sample B-703 was grown from seeds taken from a commercial lot of Cannabis Indica, No. 1728. These plants were unusual as to size and vigor, but produced an extremely low percentage of flowering tops.

Samples No. B-177 and 1493 were grown from seeds of American hemp, obtained in Lexington, Ky. The location of the plots (D and E) and the conditions of growth were practically the same as those for the foreign seed. The tops of this variety were very poor, being small and bearing a large proportion of leaves. August 17th, 1909, a sample was collected which consisted largely of leaves and stems. This was dried at room temperature and tested under No. B-177. A second sample for testing (1493) was collected, August 24th, 1909. The drug was spread thinly in

the field for twenty-four hours, during twelve of which it was exposed to direct sunlight. Drying was then completed under shelter. The following year another planting was made from the Kentucky seed under practically the same conditions. The character of the plants was the same as noted for the preceding year. A sample of the leafy tops was tested under No. B-437. No further investigation has been made of this form. The pistillate inflorescence was in all cases very small, leafy, and lacking in resin and the true Cannabis odor.

Sample No. B-693 was obtained from one extremely large and luxuriant plant. This plant was observed in a hardy border, and was tested on account of its extreme size and the very favorable appearance of its inflorescence. The exact source of the seed is not known.

In order to gain some information regarding the activity of commercial lots of American Cannabis a number of samples were purchased from drug brokers and tested physiologically. The method in brief is as follows:

Method of Preparing Doses from Crude Drugs

A 20 gramme sample of the drug in No. 60 powder is macerated with official alcohol in a small flask, which is occasionally agitated, for from 48 to 72 hours. The content of the flask is then poured into a narrow percolator. Percolation is allowed to proceed slowly, more alcohol being added as necessary. The first 90 cc. of percolate are reserved. 100 to 150 cc. more are then collected, evaporated under an air jet without heat, added to the reserved 90 cc. and made up to 100 cc. Doses of this 20% tincture are calculated, for each dog, per Kgm. of body weight. The quantity is drawn off with a pipette, evaporated without heat to an extract consistency, and made into a pill or put into a capsule.

In order to determine with certainty whether or not the active principles were completely extracted by the foregoing method, on several occasions the drug was returned to the flask and again macerated and percolated. 100 cc. of percolate were reduced, made into a pill, and given to a small susceptible dog. In no case were noticeable symptoms produced.

METHOD OF TESTING

The work is carried out on fox terriers which are kept in well ventilated, comfortable stalls. These stalls are arranged in a row, and so constructed that the dogs cannot see each other, or any object about the room which might disturb or excite them. The assays are made by one person, usually, who endeavors to maintain a thorough acquaintance with the normal movements of each animal. When a preparation is to be tested one, two or three pairs of dogs are selected which have previously been standardized against Cannabis preparations. The animals are fasted for twenty-four hours, and then to one of a pair is given (by mouth in pill or capsule) a dose of a standard preparation, and to the other a dose of the unknown. The animals are observed almost constantly during the period between one and three hours after the administration, and the results noted. After this, the animals are allowed sufficient time for recovery and complete excretion of the drug, usually about three days, and then the drugs are given again, this time in the reverse order. The next time the order is again reversed and so on. The doses are increased or diminished according to the effects previously produced, until the minimum amount of each preparation necessary to produce slight but distinct inco-ordination of the muscles is determined.

The dose of the unknown required for a given animal is compared with that required of the standard. The results on the several animals are than compared and conclusions drawn.

AMERICAN CANNABIS FROM VARIOUS COMMERCIAL SOURCES

No. of Sample	Extractive	Percentage activity compared with good Indian grown samples	Commercial Source
1032		Not more than 20%	Indianapolis
B-407		Not more than 50%	St. Louis
B-529		Not more than 40%	New York
B-824		Not more than 65%	Indianapolis
B-1034	7.98%	Not more than 50%	St. Louis
B-1040	11.08%	Not more than 50%	New York
B-1039	11.39%	Not more than 75%	New York
B-1047	13.60%	Not more than 60%	St. Louis
B-1054	15.47%	Not more than 75%	New York

The following conditions were noted in the foregoing samples:

No. B-529 contained thirty per cent of cut stems. The proportion of pistillate inflorescence was small. The odor was not pronounced or characteristic of Indian Cannabis.

No. B-1034 contained an excess of leaves, stems and seeds. The flowering tops were small.

No. B-1039 contained over fifty per cent of seeds and stems. The color of the sample was a uniform bright green. No odor of Cannabis was suggested.

No. B-1040 contained only a small proportion of seeds and stems. The drug had been compressed in such a manner as to resemble Indian Cannabis.

No. B-1047 contained a very small proportion of leaves, stems and seeds. The odor was slight and not that of Indian Cannabis.

No. B-1054 consisted of small leaves, leaf fragments, and bracts. No seeds or stems were present.

No. B-824 and No. 1032 were collected from wild plants of Cannabis sativa, and consisted largely of leafy tops. The flower clusters were extremely small, and constituted only a minor portion of the sample.

INFERIOR CANNABIS FROM FOREIGN SOURCES

That there is much inferior so-called Indian Cannabis on the market is true without doubt. It seems probable that a great deal of this has been grown, not in India, but in other places.

The following table shows the results obtained in testing some of these inferior grades. These samples were received at different times (over a period of two years), and were submitted by drug brokers upon the requests for samples of Indian Cannabis.

No. of Sample	Marks on Package	Activity compared with good Indian samples	Commercial Source
B-275	"Cannabis Indica"	Approximately 70%	Greece
B-634	"Indian Cannabis, Green Tops."	Approximately 60%	Germany
B-644	"Cannabis Herb, Madagascar."	Approximately 50%	Germany
B-660	"Cannabis Indica Herb."	Approximately 40%	Greece
B-815	"Levant."	Approximately 50%	New York
B-812	"East Indian Guaza."	Approximately 90%	London

COMMERCIAL FLUID EXTRACTS OF AMERICAN CANNABIS

Since the crude American Cannabis upon the market proved to be generally low and variable in activity, it was of interest to know whether or not commercial fluid extracts would show these same qualities. Several samples were purchased in the market and the results of these tests may be seen in the following table:

No. of Sample	Amount of extractive	Activity compared with same makers' fluid extracts from Indian grown drug
P-307	7.40%	Approximately 90%
P-308	9.86%	Approximately 100%
P-309	6.16%	Not more than 75%
P-310	6.37%	Not more than 50%
P-311 (African)	15.50%	Between 90%–100%

SUMMARY

Soil, climate and geographical location have a decided influence upon the activity of American and Indian Cannabis.

Repeated plantings from carefully selected seeds of American and Indian Cannabis have failed to yield a product testing over 65% as active as good Indian grown drug, while the majority of the plantings tested 50% and less.

Commercial samples of American Cannabis were found to vary widely in their activity. Of the samples tested none were as active as good samples of the Indian drug, and a number were not more than 50% as active.

Commercial samples from various foreign sources were supplied upon requests for samples of Cannabis Indica. None of these are equal to the Indian drug and some tested extremely low.

Commercial samples of fluid extracts of American Cannabis vary widely in their activity, some being not more than fifty per cent as active as Indian fluid extracts from the same makers.

In addition to physical and botanical characteristics, the physiological assay is of greatest importance in judging the quality of the drug. Very little dependence can be placed on the estimation of the extractive matter yielded to alcohol.

The results of this work indicate that if American Cannabis is made official, difficulty will generally be experienced in obtaining highly active lots which will compare favorably with good Indian drug.

THE INFLUENCE OF HEAT AND CHEMICALS ON THE STARCH GRAIN

By Henry Kraemer
Philadelphia, Pa.

In presenting some of the most recent observations on the starch grain, it may be well to consider for a moment the nature and origin of starch. In a way starch is one of the most remarkable substances produced by the plant. It is the first visible product formed by the chloroplastid, or chlorophyll bodies, from the inorganic substances, carbon dioxide and water. Inasmuch as sunlight seems to be necessary to bring about this transformation the process is looked upon as one which involves the converting of the sun's energy into vital energy.

The substance thus formed by the chloroplastid through the influence of sunlight, in the leaves and other green parts of plants, is known as "assimilation starch," and serves subsequently not only as a food for the plant itself but is also the source of the energy of the animal world. Assimilation starch is not stored in the cells where it is manufactured, but each night through the influence of the plant ferments the starch formed during the day is converted into a soluble form, and transported to various other parts of the plant. In some cases this soluble starch is temporarily stored in the cells of the pith, medullary rays, or bark, and has received the name of "depot starch." While some of the soluble carbohydrate is converted into fixed oils and other substances, a considerable portion of it is carried to some reserve organ, as a root, tuber, rhizome, or seed, and under the influence of a plastid similar to the chloroplastid, converted into a stable form, known as *reserve starch.*

This is the product with which we are specially concerned in the present instance. Heretofore, the minute study of the starch grain, particularly of its structure, has been of scientific interest only, but with the application of scientific methods in

nearly every department of industry, it is coming to have a practical application.

The commercial reserve starches are derived from various plants, and not only enter largely into food products but are also used for a variety of technical purposes. The grains of the reserve starches have a number of characteristic features. They vary in size, in shape, in internal structure, and also to a considerable extent in composition. The variation in composition is shown by the use of aniline stain and also by the use of iodine. By the treatment of starch with iodine solution, we may distinguish three kinds of reserve starch: (1) one which is colored deep blue, as potato and maranta; (2) one which is colored somewhat purplish, changing to cinnamon-brown, as corn and wheat; and (3) one which is colored brownish-red, as in the amylo-dextrin starches of comfrey and a few other plants.

The shape of the grains varies from polygonal to ellipsoidal, the shape being influenced by the number of grains in a cell. Under the micro-polariscope the grains are seen to be anisotropic, the polarization effects differing with the grains of the different classes. Polarizing effects are usually produced by crystals, but may be produced by substances in a condition of tension, as minute globules of glass. It should also be stated that cell walls have this same property of double refraction, and it is very likely that the substances in the starch grains, as well as in the cell wall, are crystalloidal and arranged in spherite aggregates, resembling those of inulin, a product closely resembling starch.

The theories which have been advanced regarding the structure of the starch grain, have been largely based on studies of the potato starch grain. It was originally thought to be in the nature of a globule filled with a fluid. Fritzche, Schleiden and others considered it to be made up of more or less concentric layers formed around a central or excentral point. While it may be true, as pointed out by Naegeli, that many of the reserve and glucose starch grains arise free in the cell, the view of Schimper that starch grains always develop within plastids, is generally accepted at the present time.

The internal structure of the starch grain is shown in several

ways. When starch is treated with certain chemicals, or heated with water alone to a temperature of 60 C., the grains show a series of successive changes. First, the lamellæ or layers become more distinct, and the layers appear to be made up of parallel crystal-like particles, these latter being more numerous in successive alternate lamellæ. Then as the grain swells clefts which radiate from the center are formed. Later the center of the grain becomes hollow, and when the grain has swollen to about four times its original size the outer membrane breaks and the contents are gradually dissolved.

Some striking effects are also produced when starch is carefully treated with aniline dyes. The point of origin of growth and the successive layers alternating with it take up the stains, thus again showing the distinct character of the two kinds of lamellæ making up the grains. When plant material containing mucilage is treated with aniline stains, the stain is taken up only by the cells containing mucilage, and this indicates that the lamellæ in a starch grain which take up the stains are composed chiefly of colloidal substances. From these observations it is apparent that the grains of certain of the starches, as the potato, if not of all the lamellated starch grains, are made up of two kinds of lamellæ, one rich in colloids and one rich in crystalloids. The presence of two kinds of lamellæ, at least in certain of the starch grains, and their difference of composition are further shown by the use of a weak solution of iodine, the so-called crystalloidal layers or lamellæ taking up the iodine and becoming blue.[1]

Recently I have been conducting some experiments to determine further the effects of heat upon the structure of the starch grain. When starch alone is heated to between 45 and 50 C. from 15 to 30 minutes, the lamellæ and the crystalloidal structure of the grains are brought out. The grain is so resistent that the inner structure does not appear to be lost until a temperature of over 125 C. is attained. Between 140 and 160 C. the polarization effects of the grains become faint, except in the case of potato starch, which now in addition gives chromatic effects. At 240 C. all of the grains are disintegrated except those of corn

[1]Kraemer, *Bot. Gazette*, Vol. XXXIV, Nov. 1902; *Ibid.*, Vol. XL, Oct. 1905; reprinted in *Amer. Jour. Pharm.*, Vol. 79, 1907, pp. 217-229; 412-418.

starch, the individual grains of which are of a brownish-yellow color and not perceptibly swollen. Besides the entire mass is more or less granular, while in the case of the other starches examined the charred mass is in a puffed condition.

The effects produced when starch is heated in the presence of a fixed oil, as almond oil, are of special interest. The inner structure of the starch grain is not usually apparent when it is mounted in a fixed oil, unless the starch has been previously heated to a temperature of from 80 to 160 C. When, however, a mixture of starch and oil is heated as high as 180 C. the grains still polarize light, which shows that the structure has not been altered. In other words the effects of heat on the grain are more or less neutralized by the presence of the oil. On heating the mixture up to 250 C. most of the grains still show their individual character, but no longer polarize light. They are but slightly swollen, and in the case of cassava and corn starch a central differentiated area occupies from one-half to nine-tenths of the original area of the grain.

It may be worth while to state that when starch and water in the proportion of 2 gm. of the former to 100 cc. of the latter, are heated together at a temperature of between 90 and 100 C. in a steam sterilizer seven or eight hours a day for a long period, even extending to months, dextrinization of the starch does not take place, that is, the solution still gives a blue color with iodine. Even though the operation be conducted in an autoclave under a pressure of 20 pounds for about ten hours, dextrinization is not effected. If, however, 1 c.c. of N/HCl be added to 100 cc. of water and this heated for five hours with 1 gm. of starch, the resulting solution is colored red with iodine. When the amount of the acid is reduced to .2 cc. and the mixture heated under a pressure up to 12 pounds for one hour, cassava, corn, maranta and potato starch solutions give a deep blue color with iodine, while a solution of wheat starch gives a deep purple color with iodine. If the heat be continued an hour longer, wheat starch gives a purplish red color, cassava a deep wine color, maranta and potato a light purple, while corn still gives a blue reaction with iodine.

These observations may be summarized as follows:

1. The starch grain consists of two nearly related substances: (a) a colloidal or mucilage-like substance which takes up aniline dyes, and (b) a crystalloidal or crystal-like material giving a blue color with iodine.

2. The starch grain is made up of concentric layers, one series of which contains a large proportion of crystalloids, while the alternate layers are composed mostly of colloids.

3. The polarization effects produced by starch are probably to be attributed to the crystalloidal character of the grains.

4. The starch grains retain their polarizing properties even when heated up to a temperature of 180 C., which seems very remarkable indeed.

5. At the higher temperatures the potato starch grains give chromatic effects in addition, similar to those when a selenite plate is used.

6. While heating the starch grains in water rapidly changes the structure of the grain, it is only by the addition of chemicals or ferments that dextrinization is brought about.

ARSENITES OF ALKALOIDS

By Alfred C. Mangold, Ph.G., B.C.
Brooklyn, N. Y.

I find quite a discrepancy in pharmaceutical literature in regard to compounds of alkaloids with arsenious acid.

Some of these salts being quite valuable in medicine, I undertook to investigate their constitution, making a number of experiments according to different methods, using both the cinchona and the strychnos alkaloids.

QUININE ARSENITE

$(C_{20}H_{24}N_2O_2)_3\ H_3\ as\ O_3 + 4H_2\ O$

The formula for making this preparation as given in the National Standard Dispensatory 1909 reads:

"The salt is prepared by boiling 5 parts of silver arsenite and 11.3 parts of quinine hydrochloride with 100 parts of 70% alcohol under a reflux condenser until the transposition is complete, filtering, and crystallizing the salt from the clear filtrate. Silky white needles are formed, sparingly soluble in cold water, soluble in about 150 parts of boiling water, in 15 parts of cold and 6 parts of boiling alcohol, in 8 parts of chloroform, in 25 parts of ether and in 20 parts of benzene."

The salt contains 83.08% of quinine and 8.45% of arsenious acid (AS_2O_3).

QUININE ARSENITE

Experiment No. 1

This experiment was made according to the formula in the National Standard Dispensatory 1909, using:

Quinine hydrochloride	50.00 gm.
Silver arsenite	22.12 gm.
Alcohol	442.00 gm.

After boiling the mixture according to directions given, filtering and allowing the liquid to stand and crystallize for 12 days,

a white amorphous substance was formed, adhering to the sides of the dish, due to the evaporation of the liquid.

On analysis this substance was found to contain:

	Theory	Found
Quinine alkaloid	83.08%	63.30%
Arsenious acid H_3ASO_3	10.76%	34.57%
Water	6.16%	1.60%
	100.00	99.47%

This analysis shows that the quinine and arsenious acid did not combine to form a true salt.

QUININE ARSENITE

Experiment No. 2

In this experiment the proportions as given in the National Standard Dispensatory were used, but the mixture was boiled for 4 hours continuously after the transposition had taken place, the silver chloride which had formed was reduced.

After filtering the liquid and allowing it to stand for three days, crystals were formed. I found these crystals to be quinine arsenate.

ANALYSIS

	Theory	Found
Quinine alkaloid	69.38%	70.00%
Arsenic acid	15.19%	14.32%
Water	15.43%	15.00%
	100.00%	99.32%

The formation of arsenic acid in this experiment was no doubt due to the reduction of the silver, which oxidized the arsenious acid to arsenic acid, which combines quite readily with alkaloids to form salts.

QUININE ARSENITE

Experiment No. 3

This experiment was made by boiling together molecular proportions of quinine alkaloid and arsenious acid in water for

several hours, filtering and allowing the clear hot liquid to stand and crystallize.

Soft white crystals were formed.

On examining these under microscope I found them to be soft needle-shaped crystals, having the same form as alkaloid quinine when crystallized from a hot aqueous solution.

These crystals contained 99% of quinine alkaloid, this showing that quinine alkaloid when boiled with arsenious acid in water does not combine to form a chemical compound.

QUININE ARSENITE

Experiment No. 4

This experiment was made by adding a hot aqueous solution of potassium arsenite to a hot aqueous solution of quinine sulphate, using molecular proportions.

On pouring the potassium arsenite solution into the quinine sulphate solution, a white amorphous substance was immediately formed. The solution containing the amorphous substance crystallized after standing two days, soft, white, needle-shaped crystals being formed.

The crystals and amorphous substance were transferred to a filter and washed well with cold water and dried.

On examining this substance I found the amorphous sediment to be quinine alkaloid and the crystals quinine sulphate.

ANALYSIS

	Theory
Quinine alkaloid	83.08%
Arsenious acid	10.76%
Water	6.16%
	100.00%

	Found
Quinine sulphate	63.71%
Quinine alkaloid free	32.06%
Water	4.00%
	99.77%

QUININE ARSENITE

Experiment No. 5

This experiment was made according to the U. S. Dispensatory 1900 which states to prepare the salt by dissolving arsenious acid in water with enough alkali to make the solution neutral, adding abut five times its weight of silver nitrate, washing the precipitated arsenite of silver, drying and mixing with three times its weight of quinine hydrochloride, digesting the mixture for a day with 70% alcohol, filtering and allowing the filtrate to evaporate spontaneously.

The salt is crystalline, soluble in alcohol, chloroform and ether, but sparingly so in water.

I found in making this experiment that after the liquid had been standing for 5 days a white amorphous looking substance had formed around the sides of the dish; this was removed, dried and examined.

ANALYSIS

	Theory	Found
Quinine alkaloid	83.08%	92.50%
Arsenious acid	10.76%	3.87%
Water	6.16%	3.00%
	100.00%	99.37%

This analysis shows that the substance formed in this experiment was not a true salt of quinine arsenite.

CINCHONIDINE ARSENITE

Experiment No. 6

This experiment was made by boiling together 15 gm. of cinchonidine hydrochloride with 6.8 gm. of silver arsenite in 225 gm. of 70% alcohol under a reflux condenser until the transposition was complete, filtering and allowing the liquid to stand in a cool place to crystallize.

After the liquid had been standing for 24 hours hard white crystals were formed; these crystals were placed in a funnel to allow the liquor to drain off, after which they were dried and examined.

ANALYSIS

	Theory	Found
Cinchonidine alkaloid	88.73%	97.70%
Arsenious acid	11.27%	1.25%
	100.00%	98.95%

This analysis shows that arsenious acid does not combine with cinchonidine to form a true salt.

CINCHONINE ARSENITE

Experiment No. 7

This experiment was made by boiling together 15 gm. of cinchonine hydrochloride with 6.8 gm. of silver arsenite in 375 gm. of 70% alcohol under a reflux condenser until the transposition was complete, filtering and allowing the liquid to stand in a cool place to crystallize; after standing over night hard white crystals were formed.

The crystals were placed in a funnel to allow the liquor to drain off, after which they were dried and examined.

ANALYSIS

	Theory	Found
Cinchonine alkaloid	88.73%	99.80%
Arsenious acid	11.27%	.19%
Water		.50%
	100.00%	100.49%

This analysis shows that cinchonine does not combine with arsenious acid to form a true salt.

QUINIDINE ARSENITE

Experiment No. 8

This experiment was made by boiling together 15 gm. of quinidine hydrochloride with 6.2 gm. of silver arsenite in 100 gm. of 70% alcohol under a reflux condenser until the transposition was complete, filtering and allowing the liquid to stand in a cool place to crystallize; after standing over night find white crystals were formed.

The crystals were placed in a funnel to allow the liquor to drain off, after which they were dried and examined.

ANALYSIS

	Theory	Found
Quinidine alkaloid	89.57%	88.20%
Arsenious acid	10.43%	.81%
Water		12.00%
	100.00%	101.01%

This analysis shows that arsenious acid does not combine with quinidine to form a true salt.

BRUCINE ARSENITE

Experiment No. 9

This experiment was made by boiling together 15 gm. of brucine hydrochloride with 5.2 gm. of silver arsenite in 150 gm. of 70% alcohol under a reflux condenser until the transposition was complete, filtering and allowing the liquid to stand in a cool place to crystallize; after standing for two days long, fine crystals were formed.

The crystals were placed in a funnel to allow the liquor to drain off, after which they were dried and examined.

ANALYSIS

	Theory	Found
Brucine alkaloid	90.37%	82.00%
Arsenious acid	9.63%	5.40%
Water		13.50%
	100.00%	100.90%

This analysis shows that brucine does not combine with arsenious acid to form a true salt.

STRYCHNINE ARSENITE

Experiment No. 10

This experiment was made by boiling together 15 gm. of strychnine hydrochloride with 6.1 gm. of silver arsenite in 500 gm.

of 70% alcohol under a reflux condenser until the transposition was complete, filtering and allowing the liquid to stand in a cool place to crystallize; after standing 24 hours hard white crystals were formed.

The crystals were placed in a funnel to allow the liquor to drain off, after which they were dried and examined.

ANALYSIS

	Theory	Found
Strychnine alkaloid	88.84%	97.73%
Arsenious acid	11.16%	1.25%
Water		.50%
	100.00%	99.48%

This analysis shows that strychnine does not combine with arsenious acid to form a true salt.

I found in all the experiments made that the arsenious acid found on analysis was not combined with the alkaloid, but simply adhering to it, showing that arsenious acid does not form salts with these alkaloids, while arsenic acid as stated before, forms well crystallizing definite compounds.

PREVENTION OF EMULSIFICATION IN EXTRACTIONS BY IMMISCIBLE SOLVENTS

By G. H. Meeker, Ph.D.
Medico-Chirurgical College, Philadelphia, Pa.

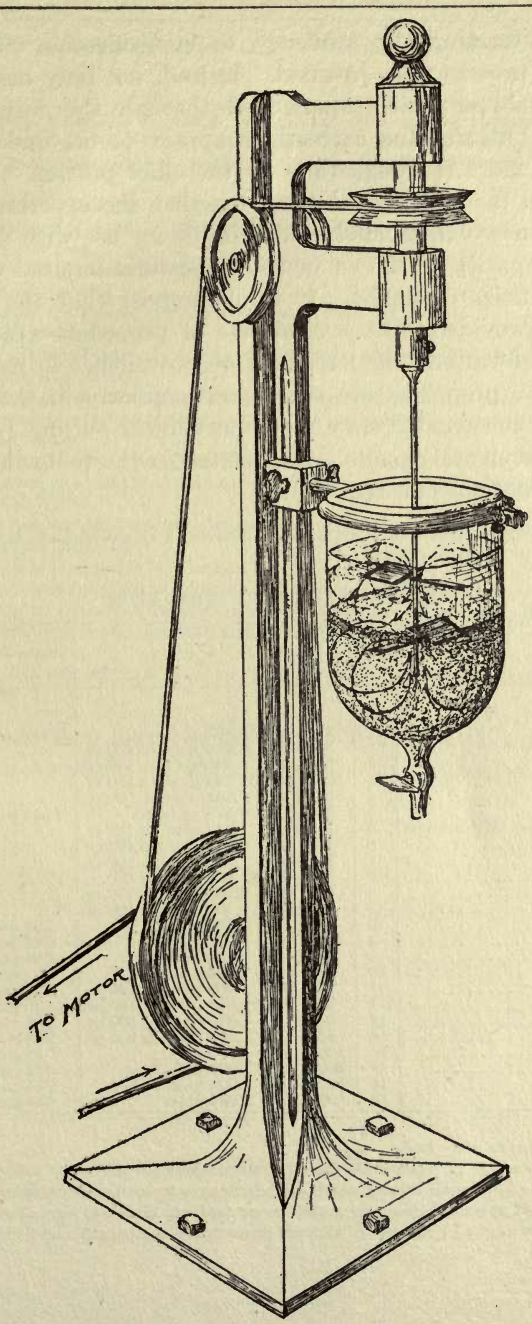
One of the most important processes employed in analytic chemistry is the process referred to on page 578 of the 8th Revision of the U. S. P. as the "shaking-out" process. This process is usually conducted in a separatory funnel commonly called a "separator." Generally speaking, the purpose of the process is, by a special method of solution, to extract substances from one liquid by agitating that liquid with a second liquid immiscible with it. Substances are in this way caused to pass from solution in one liquid to solution in the other. In most cases ether, chloroform, petroleum ether, benzol, ethyl acetate and amyl alcohol, singly or admixed, are employed on the one hand — whereas neutral, acid and alkaline aqueous solutions are employed on the other hand; and alkaloids constitute the most important class of dissolved substances. The process of shaking-out is too well known to call for further comment here. In the process of shaking-out the analyst has constantly to contend with a serious practical difficulty, namely, the tendency toward the more or less extensive formation of emulsions which render the plane of contact between the heavier and lighter liquid indistinct, and mechanically prevent ready separation of the two liquid layers. Various artifices have been proposed for minimizing or preventing the foregoing difficulty, or for breaking up the emulsions after their formation, but it is believed that the apparatus herein described provides decidedly the most effective remedy yet suggested in the premises. The purpose of this apparatus is *to bring, automatically, continually renewed surfaces of the two liquids in the separator into contact with each other without an inter-agitation productive of emulsification.*

The apparatus is readily understood by an inspection of the accompanying etching. The apparatus consists of a cylindrical separator (or any other suitable vessel according to the analyst's

convenience) containing the two liquid layers. A vertical shaft, having its axis coincident with the axis of the separator, dips into the liquids. This shaft carries adjustably near its lower extremity a two-bladed propeller, which propeller is entirely immersed in the lower liquid layer. A similar propeller, with reversed thrust, is adjustably attached to the shaft above the lower propeller at such a height as to be entirely immersed in the upper liquid layer. The propeller shaft is further equipped with a pulley located conveniently above the separator and is rigidly supported in a suitable bearing upon a horizontal arm attached to a vertical support, which is firmly attached to a bed plate. The bed plate rests upon the work table and serves as a foundation for all parts of the apparatus except the motor. Provision is made for belting the propeller-shaft pulley to a motor — an electric motor being the most convenient kind. The mechanical parts should be so heavily, rigidly and accurately constructed that the movements of the liquids are truly circulatory and free from any excessive beating action due to wabbling of the propeller shaft or unbalancing of the propellers. The immersed portion of the shaft and the propellers should be made of glass, gold or platinum if necessary to resist chemical wear; but in the experimental apparatus pure nickel has been employed with entirely satisfactory results.

The shaft is made to rotate in that direction which will cause the lower propeller to drive the lower liquid layer, which lies immediately above it, upward — when, because of the reversed thrust of the upper propeller, that layer of the upper liquid which lies beneath the upper propeller will be set into downward motion. When the shaft revolves, therefore, a circulatory, convolute motion is communicated to each of the liquid layers, causing a continual radial renewal of the surfaces of contact between the two liquids. Assuming that the propellers are rotated at the optimum speed, the liquid layers are continually renewed by intra-agitation of the two liquids not of a nature to cause them to possess sufficient inter-agitation to produce emulsification.

The apparatus has but recently been tested in the extraction of alkaloids, and report of its extensive use cannot at this time be made. Sufficient has been done, however, to show that the expectations that the apparatus would be effective as a separator


To Motor

and in overcoming the tendency to emulsification during the separatory process were justified. Indeed, not only has no difficulty whatsoever been experienced through the formation of emulsions, but also the extractions appear to be, under correct conditions, more thorough than by the older process. The only difficulty so far experienced has been that the experimental apparatus as now constructed, and suitable for use with separators having a capacity of 125 cc. or 250 cc., cannot be used with very small quantities of liquid. It would appear that the operator should be provided with several sets of propellers available for separators of various diameters. For convenience in making the drawing, the propellers are shown rectangularly in the etching. As actually constructed they are of curvilinear outline.

The experimental results are exhibited in the following tabulations, explanations and remarks:

Table exhibiting 38 experimental extractions, involving 6 alkaloids, made in the course of an investigation of the practicability of

THE "NON-EMULSIFYING SEPARATOR"

I	II	III	IV	V	VI
.....	1	...	..	.....	.....
Hydrastine.....	2	36	30	2.697 per cent	2.660 per cent
Hydrastine.....	3	36	30	1.818 gms. in 100 cc.	.....
Hydrastine.....	3	36	30	1.828 gms. in 100 cc.	.....
Hydrastine.....	4	36	30	2.184 gms. in 100 cc.	.....
Hydrastine.....	4	36	30	2.144 gms. in 100 cc.	.....
Hydrastine.....	5	36	15	2.518 per cent	2.542 per cent
Hyoscyamine, etc. (Mydriatics)	6	36	30	.012 gms. in 100 cc.	.....
Hyoscyamine.....	6	36	30	.012 gms. in 100 cc.	.....
Hyoscyamine.....	7	36	30	.009 gms. in 100 cc.	.....
Hyoscyamine.....	7	36	30	.009 gms. in 100 cc.	.....
Sanguinarine.....	8	36	30	2.580 gms. in 100 cc.	.....
Sanguinarine.....	8	36	30	2.100 gms. in 100 cc.	.....
Colchicine.....	9	55	5	.389 gms.	.388 gms.
Colchicine.....	9	55	5	.327 gms.	.363 gms.
Aconitine.....	10	55	5	.358 gms.	.384 gms.
Aconitine.....	10	55	5	.346 gms.	.371 gms.
Strychnine.....	11	36	20	1.009 gms. in 100 cc.	1.205 gms. in 100 cc.
Strychnine.....	11	36	20	1.023 gms. in 100 cc.	1.185 gms. in 100 cc.
Strychnine.....	12	55	5	.876 gms. in 100 cc.	.969 gms. in 100 cc.
Strychnine.....	12	55	5	.896 gms. in 100 cc.	.956 gms. in 100 cc.
Strychnine.....	13	100	10	.025 gms.	.023 gms.
Strychnine.....	14	100	10	.024 gms.	.023 gms.
Strychnine.....	14	100	10	.024 gms.	.023 gms.
Strychnine.....	15	100	10	.023 gms.	.023 gms.

Meaning of columns in above table:

I, alkaloid extracted; II, remark number; III, revolutions per minute of propeller shaft of machine; IV, time in minutes during which propellers were in motion; V, amount of alkaloid recovered by use of non-emulsifying separator (in no case was there any sign of emulsification when machine was used); VI, amount of alkaloid recovered in comparative test, using the com-

mon "shaking-out" process of extraction. (The usual difficulties due to emulsification were encountered.)

Remarks:

1. Ten minutes of machine extraction with the propellers making at least one hundred revolutions per minute appear to be satisfactory conditions. Cases of incomplete extraction in the table seem, obviously, due to insufficient speed of propellers or brevity of time of extraction.

2. U. S. P. assay of a sample of hydrastis.

3. U. S. P. assay of a sample of fluid extract of hydrastis. Actual weight of alkaloid has been multiplied by twenty.

4. Same as 3 after concentrating the fluid extract.

5. Like 2, using root only.

6. U. S. P. assay of a sample of tincture of hyocyamus.

7. Like 6, but another sample.

8. Technical method of assay of a sample of fluid extract of sanguinaria, U. S. P. Laboratory standard is 2.5 grams in 100 cc.

9. U. S. P. assay process for fluid extract of colchicum seed, applied to 10 cc. of an aqueous solution of presumably pure colchicine containing .04 gm. colchicine. Actual weight of colchicine multiplied by ten.

10. Like 9; but applying to fluid extract of aconite and to aconitine.

11. U. S. P. assay of a sample of fluid extract of nux vomica. Actual weight of alkaloid has been multiplied by ten.

12. Like 11; but another sample. Aqueous solutions diluted when necessary to obtain sufficient bulk for the propellers.

13. U. S. P. assay process for fluid extract of nux vomica applied to 10 cc. of a solution of presumably pure strychnine in dilute sulphuric acid, containing .025 gm. strychnine.

14. Like 13; but 10 drops of olive oil were added. The olive oil caused no difficulty whatsoever in the machine process, but emulsification and much consequent delay occurred in the "shaking-out" process.

15. Like 14; but with addition of 10 cc. fluid extract of taraxacum instead of olive oil. Behavior as in 14.

I wish to express my indebtedness to Eugene L. Maines, Ph.C., for his most valuable aid throughout the investigation.

THE POTENCY OF FIRST YEAR CULTIVATED DIGITALIS LEAVES AS INDICATED BY PHYSIOLOGICAL ASSAY

By F. A. Miller, B.S., and W. F. Baker, B.S., M.D.
Indianapolis, Ind.

The supply of many valuable medicinal plants once so abundant from natural sources is rapidly becoming exhausted. This has been brought about by the destruction of the forests; the devotion of more and more of the waste lands to agriculture, and the destructive methods of harvesting without any thought or care to preservation. The supply was ample and little effort was made toward cultivation. Where attempts were made to introduce medicinal plants into other countries, soil and climatic conditions were often unfavorable. Many plant forms mature very slowly and several years must pass before the drug can be marketed. Possibly from this arose the belief that cultivated plants were less valuable than wild ones. The small returns to be had and the limited demand for the product has been responsible for the undevelopment of this form of industry.

In view of the advance now being made in plant culture and improvement it is entirely within the realm of possibility that the desired constituents of drug plants can be increased and undesirable ones decreased or eliminated as is being done with many cultivated forms. In the case of some drugs, the active principles of which can be more or less closely paralleled by synthetic preparations, it may be questionable as to the advisability of expending much energy upon their cultivation.

The forms which contain neutral principles, glucosides, etc., cannot be so closely paralleled. In this class digitalis easily stands first. One of the factors in the way of its successful cultivation has been the belief that wild plants were more potent, and this has been furthered by the Pharmacopeias of various countries. Some of these Pharmacopeias now admit cultivated leaves, but all require the second-year leaves at the time of flowering. This

requirement appears to be founded more upon tradition than as a result of scientific investigation. This is a question of considerable economic importance. A larger quantity of leaves can be obtained from first year plants, and if it can be conclusively proven that first year leaves are as therapeutically active as the second, it will greatly shorten the period and the cost of production.

From a review of the literature it appears that very little work has been done to determine the relative potency of first and second year leaves.

Duffield[1] in 1869 using chemical methods found American leaves superior to English and these in turn superior to German. Edmunds[2] assayed biologically, three U. S. P. tinctures made from English leaves and three from German leaves and found them to give values of 8-18-25 and 11-20-29 respectively. Ott[3] pointed out that Bohemian leaves were much more toxic than others.

Focke[4] found cultivated leaves to be 50% less active than leaves from wild plants. Allen's English leaves are garden grown and are recognized as quite active.

Hart[5] found first year's leaves to be 20% more toxic than second year. Hale[6] reports some assays of first year garden-grown leaves, one sample grown at Arlington, Va., in 1907, a second in 1909, and a third sample grown at Madison, Wis., in 1908. Each lot of leaves was reduced to a No. 60 powder and made into tinctures according to the U. S. P. VIII. The M. L. D. per gram body weight by the one hour frog heart method, for Arlington, 1907, was 0.0050 cc.; for the 1909, 0.0050 cc.; and for the Wisconsin, 1908, 0.0055 cc. These were compared with a tincture made at same time from selected English leaves of second year's growth. The M. L. D. for this was 0.0070 cc. He further reports a comparison of a sample of first year garden-grown with a sample of wild-growing second year leaves from

[1]Duffield: Am. Jr. of Pharm. 1869, XLI, 55.
[2]Edmunds: Jr. A. M. A. 1907, XLVII, 1744.
[3]Focke: Arch. der Pharm. 1903, CCXLI, 128.
[4]Hale: Bull. No. 74, Hyg. Lab. U. S. Pub. Health and Marine Hospital. Service.
[5]Hart: Pharm. Jr. and Tr. 1908, XXVI, 440.
[6]Ott: Verhandl. d. Cong. f. innere Med. Wiesb. 1901.

Seattle, Wash. The first year leaves assayed 0.0060 and the second year leaves 0.0085.

In view of these statements and of the approaching pharmacopeial revision it has been deemed advisable to publish the results of some assays of samples of first year cultivated leaves and compare them with samples of selected commercial leaves. The samples tested were taken from plants grown from seed purchased of Henry A. Dreer of Philadelphia. The botanical source is Digitalis gloxineaeflora mixed. It is a well-known fact that this form is a cultivated and improved gloxinia-like strain of the official Digitalis purpurea L. It is probably one of the oldest of the cultivated varieties of the genus Digitalis and for this reason should represent the average conditions which are to be expected in cultivated forms of this group. The seeds were planted June 10th, 1911 and soon after germination were transplanted to two-inch flower pots. They were retained in these until the latter part of August of the same year when they were transplanted to a bed in the conservatory maintained at this laboratory.

The bed was filled with a mixture of equal parts of clay loam and decayed vegetable substance with an admixture of a small amount of sand. The plants were grown continuously in this situation without the addition of any plant food until the second of February, 1912, when the leaves were collected for testing. The plants at this time appeared as large rosettes of luxuriant leaves. They were in perfect condition and represented a considerable range in leaf variation as noted in about fifty plants. These plants, including the three tested, have been under observation and continuous cultivation throughout their entire existence and have responded perfectly to regular methods of cultivation. The leaves which were collected for testing consisted of about an equal mixture of fully developed and half-grown leaves. These when collected were designated as samples B-994, B-995 and B-996. They were placed in a drying oven and the temperature slowly raised to 100°C. Drying was considered complete at the end of twenty-four hours. During an interval of twelve hours the temperature was lowered to 60° C. The leaves after being dried were reduced to a No. 60 powder and exhausted with

75% alcohol. At same time and by same method tinctures were made from samples of commercial leaves designated as No. 1, 2 (cultivated) and 3. The alcoholic strength was reduced to 25% before testing. Ten cc. of these tinctures represented 1 gm. of drug. These samples were all assayed at same time by the one hour frog heart method at 20°C.

The results were as follows:

Sample	M. L. D. per gm. body weight.
B-994	0.0050 cc.
B-995	0.0040 cc.
B-996	0.0030 cc.
No. 1	0.0040 cc.
No. 2 (cultivated)	0.0030 cc.
No. 3	0.0025 cc.
Ouabain 1:10000	0.00000045 gm.

These results, although not conclusive, indicate that leaves from horticultural varieties are by no means inactive; that leaves collected prior to the flowering period may be nearly or quite as active as second year leaves from the wild plants and that conservatory conditions do not materially lessen the activity. There also appears to be a marked variation in the activity of the individual plants. This condition if existent in commercial digitalis leaves may account in part at least for the variation that has been found to exist, both in the crude drug and its preparations. Whatever influence this individual variation may have upon the character and quality of the drug, or whether it may ever be utilized in bringing about greater uniformity, are matters of conjecture. This condition of variation, however, suggests a broad field for further investigations in the improvement of medicinal plants. If the active constituents of digitalis were found to be of an inherent nature a practical application of the methods of plant selection and breeding would be possible for the purpose of improvement. In this connection, it would be of great importance if the leaves of first year plants of cultivated varieties could be utilized. These forms have already passed through the experimental stage which is always necessary to the successful introduction of any plant forms and would thus re-

spond more readily to methods of breeding. The preliminary nature of this work, however, will not permit of a full discussion of the possibilities in drug plant improvement. It is sufficient to say that work of this nature has been undertaken in connection with the further testing of the many different species and varieties of digitalis. Seeds of these forms have been obtained not only from all the prominent sources of this country but also from those of England, Germany and Japan.

Individual plants of the various forms from these sources will be tested at different periods of growth and at different seasons. The three plants herein considered will be continued under cultivation and again tested at the time of flowering. At this time these plants will be inbred and the seeds collected separately. The following year the progeny will be grown and tested separately for relative values and uniformity.

THE ALKALOIDAL CONTENT OF INDIVIDUAL PLANTS OF DATURA STRAMONIUM L. AND DATURA TATULA L.

By F. A. Miller and J. W. Meader
Indianapolis, Indiana

The examination of individual plants of *Datura Stramonium* L. and *Datura tatula* L. for their total alkaloidal content has been undertaken for two reasons; first, as a means of following the effects of prolonged cultivation upon the percentage of alkaloids, and second as a means of selecting high yielding individual plants. These high yielding plants are intended to serve as parents of future generations from which continued selections can be made. In these selections the object is to develop pure strains, which, it is hoped, will exhibit a racial improvement in relative alkaloidal value over wild plants or those cultivated without any effort toward improvement. It has been shown[1] that the application of certain nitrogenous fertilizers to members of the solonaceous family, and especially belladonna, will cause a perceptible increase in the alkaloidal content. This increase, however, does not represent an improvement as understood by plant breeders. It is environmental and thus only temporary. What is most needed to further the advancement of drug growing is a permanent improvement of the more valuable medicinal plants. This may be accomplished through the application of special methods of breeding as now practiced by plant breeders, horticulturists and florists.

Little has been done on the cultivation and improvement of stramonium except in the case of a few species, which are grown for decorative purposes. Experimental plots of several species have been grown, but apparently have not been continuously cultivated. As to improvement, there is no evidence of any attempts having been made, except that by Meyer[2] to hybridize *Datura Metel* L. and *Datura Stramonium* L.

[1]Chevalier: Comptes Rendus. 1910, 150, 344.
[2]Meyer: Arch. der Pharm. 1905, 243, 304.

Members of the genus other than *Datura Stramonium* L. have been reported upon favorably and may be found through further investigation to be equally valuable. *Datura Metel* L. has been shown by Schmidt[1,2] to contain as much hyoscyamin as *Datura Stramonium* L., also that *Datura alba* Nees which is grown in China, India and southern Europe for decorative purposes contains hyoscyamin and some atropin in the seed. Hesse[3] also found considerable amounts of alkaloid in this species. Peinemann[4] is quoted as having found more alkaloid in the leaf, seed and root of this species than in the corresponding parts of *Datura Stramonium* L. Kircher[5] examined *Datura Metel* L., *Datura quercifolia* and *Datura arborea* L., finding in the leaves and seeds of quercifolia an average of 0.418% and 0.292% of alkaloids respectively. He also noted the presence of alkaloids in *Datura Metel* L. and *Datura arborea* L.

The desirability of cultivating and improving *Datura Stramonium* L. is indicated by an examination of commercial conditions. These conditions as enumerated below indicate a wide range of variations in percentage of alkaloids from year to year and from different geographical sources. Feldhaus[6] in the examination of twenty-five commercial samples from various sources notes a variation of from 0.211% to 0.495% with an average of 0.328%. In 1901 he found an average of 0.476%, while in 1902 the average was only 0.337%. Farr and Wright[7] state that in 1906 they found a maximum of 0.30% and a minimum of 0.12%, average 0.22%. They quote Kordes as having found 0.20% and Umney 0.39% to 0.44%. Smith, Kline and French[8] in 1908 report on sixteen assays which indicate a range of from 0.25% to 0.37%, nine of these being below the United States Pharmacopial standard. In 1911[9] they again report on fifteen assays ranging from 0.22% to 0.35%, three of these being below standard, seven

[1]Schmidt: Arch. der Pharm. 1905, 243, 303.
[2]Schmidt: Arch. der Pharm. 1910, 248, 641.
[3]Hesse: Pharm. Jr. 1900, 64, 250.
[4]Peinemann: Handelsbericht von Gehe & Co. 1896.
[5]Kircher: Arch. der Pharm. 1905, 243, 309.
[6]Feldhaus: Arch. der Pharm. 1905, 243, 328.
[7]Farr & Wright: Pharm. Jr. 1906, 76, 310.
[8]Smith, Kline & French: Anal. Rept. 1908, 36.
[9]Smith, Kline & French: Anal. Rept. 1911, 42.

not exceeding 0.28% and five running above 0.30%. Vanderkleed[1] in 1907 reported on nineteen assays ranging from 0.15% to 0.62%. Again in 1908[3] he considers twenty-five samples of which the lowest was 0.13%, highest 0.51%, average 0.34%. Twenty-one of these were above and four below the official standard. Hankey[4] examined lots which ran as low as 0.14%, with an average of only 0.25% for the best. Puckner[2] gives a variation of from 0.13% to 0.45% as found in sixteen samples. Average results from the Lilly laboratories for the past five years indicate an annual variation and a recent annual decrease in alkaloidal content: 1907, 0.34%; 1908, 0.40%; 1909, 0.38%; 1910, 0.32%; 1911,0.25%. The foregoing data indicates the desirability of a *uniform high yielding plant.*

The objects of the present investigation have already been stated. The species used were *Datura Stramonium* L. and *Datura tatula* L., both native of this country. The plants of *Datura Stramonium* L. were grown from seed purchased in the London market. This seed was not absolutely pure, as one *Datura tatula* L. plant appeared in the experimental plot from the first planting. The *Datura tatula* L. plants used in the experiment were transplanted from a vacant lot in Indianapolis. The two forms were grown under the same conditions on soil consisting of stiff clay loam. Cultivation was frequent and continued until mature seed could be obtained.

The samples of leaves for assay from the *Datura tatula* L. plants were collected August 17th, 1910. At this time the plants bore mature but unripe seed pods, open flowers and numerous buds. August 30th the plot of *Datura stramonium* had reached a corresponding stage of maturity and samples were collected on this date. In both cases, individual plants of vigorous growth were selected and numbered. The samples as removed from these individuals were given the corresponding number of the plant and retained separately. Later, mature seeds were collected from the same plants. The number of leaves removed was in no instance so great as to interfere with normal growth.

[1]Vanderkleed: Proc. Penn. Pharm. Ass. 1907, 90.
[2]Vanderkleed: Proc. Penn. Pharm. Assocn. 1908, 88.
[3]Hankey: Am. Drug. 1907, 50, 9.
[4]Puckner: Proc. Am. Pharm. Ass. 1906, 440.

The samples were thoroughly cured at room temperature and stored in paper bags until one year later, when they were assayed. The individual plants assayed as follows:

No. B-979	*Datura Stramonium* L.	0.47%
No. B-980	*Datura Stramonium* L.	0.55%
No. B-981	*Datura Stramonium* L.	0.52%
No. B-982	*Datura Stramonium* L.	0.46%
No. B-983	*Datura tatula* L.	0.63%
No. B-984	*Datura tatula* L.	0.65%
No. B-985	*Datura tatula* L.	0.47%

The process of the United States Pharmacopœia for the assay of stramonium was used, taking liberal amounts of solvent in all cases, and with the exception that $\frac{N}{20}$ hydrochloric acid was substituted for $\frac{N}{10}$ sulphuric acid in titrating.

It will be noted from the foregoing figures that there is a marked variation in the total alkaloidal percentage of individual plants. Attention has been called to the variation in commercial stramonium for different years and for different geographical sources. Little is known, however, upon the behavior of the percentage of alkaloids in individual plants. True[1] has found that individual belladonna plants vary from 0.2% to 0.7% in total alkaloids. The extremes as found for *D. Stramonium* L. are maximum 0.55%, minimum 0.46%, and for *D. tatula* L., maximum 0.65%, minimum 0.47%.

Datura tatula L., a species very closely related botanically to the official *Datura Stramonium* L., indicates a much higher alkaloidal percentage than the U. S. Pharmacopœial species. Both forms considered, likewise show a higher percentage than any commercial drug examined during the past five years, the maximum for this period at the Lilly laboratories being 0.40%, minimum, 0.25% and average 0.33%. The results obtained also demonstrate that continuous cultivation does not interfere with the natural formation of a high percentage of alkaloids.

[1]True: Oil, Paint & Drug Reporter 1911, 80, 29.

The investigation of individual plants from this group is to be continued upon the same plan. Seeds were collected from the plants assayed and those from the highest and lowest yielding plants will be planted and individuals again tested. In one case the object is to bring about an increase in the percentage of alkaloids, while in the other it is to decrease this percentage.

BELLADONNA. THE EFFECT OF CULTIVATION AND FERTILIZATION ON THE GROWTH OF THE PLANT AND ON THE ALKALOIDAL CONTENT OF THE LEAVES

By Francis Ransom, F.C.S., and H. John Henderson

Hitchin, England

The following paper is the result of work undertaken in response to a resolution passed at the last meeting of the Congress "that it is desirable that an international enquiry should be instituted with a view to securing greater uniformity in the commercial supplies of potent drugs and the means for determining the same."

Belladonna being a drug known to vary greatly in activity, it was thought desirable to initiate an investigation to determine the extent to which varying conditions of cultivation affect the alkaloidal content of the plant.

A paper on a similar subject was published by J. Chevalier (Comptes rend. 1910, 150, 344) two years ago. It is here stated that the use of nitrates with farmyard manure has the effect of doubling the amount of alkaloids in the dried leaves of Belladonna, Hyoscyamus and Stramonium. Potassic manures and phosphates are stated to have no influence on the amount of alkaloids.

A problem which has for its solution a method for increasing or regulating the amount of remedial or toxic principle of whatever kind in medicinal plants is affected by diverse and varying factors, controllable and uncontrollable, and is a question of such intricacy and magnitude as to require the expenditure of much time and money to make experiments in but a small corner of this field of research.

The variations of the soil are sufficient to mislead the unwary into making rash forecasts as to the effect which this or that element, or group of elements, will produce when applied to the growing plant. The mangold plots at Rothamsted were a case in point. A soil, which contains potash in a form not readily assimilable

by the plant, is dressed with sodium nitrate with most satisfactory results, and the conclusion is drawn that the nitrate is directly responsible for them; certainly it is responsible for them, but in an indirect manner not readily appreciated, for the sodium nitrate has made the potash which was unavailable, available, that which was unworthy, worthy, and owing to double reaction, complex most probably in the intermediate stages, the potash enters into the plant with striking effect. This effect is attributed to the nitrate, and it is only when nitrate fails to produce the same effect on another field situate perhaps on the same farm, that a suspicion dawns upon the mind as to the accuracy of the conclusion.

When effects such as these are produced upon different fields on the same farm, nay, in the same field itself, it is evident how wrong conclusions may be drawn from experiments carried out in different countries. Even on the same farm the aspect of the ground must be considered. Is it bleak and bare, lying exposed to the cutting northeast wind? Does it lie under the warm lee of a sheltering slope, fanned by the southern breeze? Is the texture of the soil firm or friable, heavy clay or light loam? Is the soil warm and moist, or cold and wet? These are the factors that will play as large, and what is more likely, an even larger part in the physiology of the plant and its metabolism than the manures with which it is artificially fed.

The situation, texture, drainage, and all that go to make land in good heart are the controllable factors; but to harness the wind, screen the sun and drench drooping and dispirited vegetation when parched by drought appertain to the regions of necromancy and not to sober science; these being the uncontrollable factors.

The results here presented must, therefore, be viewed as the tentative results which further experience may modify. They are but the first links in a chain which must of necessity be forged slowly. The plots are new and our knowledge of them is very imperfect; they are probably unequal and time alone will reduce them to a level plane for perfect comparison. A mechanical analysis of the soil by the method described by Dr. Hall in his book "The Soil" (page 50) gave the following results, five samples being taken and mixed to form two. The material which failed

to pass through a 3mm. sieve was of two kinds, viz., large stones, pieces of chalk, etc., and a finer material consisting of aggregated lumps of very hard earth, little stones and hard nodules of chalk (together with vegetable debris) in both cases.

The weight of large stones in sample 1 before washing was 3 lbs. 3 ozs., after washing, 2½ lbs.; fine stones before washing 8 ozs., after washing 7¼ ozs. The weight of air-dry fine earth was 21 lbs. 11 ozs. The weight of large stones in sample 2 was 2 lbs. 9 ozs., and small stones 11¾ ozs.; after washing 2 lbs. 60 grs. and 4 ozs. 180 grs. respectively; and the weight of the air-dry earth was 13 lbs. exactly. The air-dry fine earth effervesced strongly with $\frac{N}{5}$ HCl in both cases.

Summary of results of analysis of air-dry fine earth.

	Sample 1	Sample 2
Fine gravel	4.46	3.13
Coarse sand	36.48	30.
Fine sand	14.00	14.3
Silt	8.37	7.25
Fine silt	8.12	8.2
Clay	3.94	4.2
Soluble matter	19.77	24.0
Humus	3.44	4.1
	98.58	95.18

The plots are eleven in number and are situated in the old herb garden which adjoins the distillery of W. Ransom & Son, Hitchin, near London. The seed employed was all obtained from the same source, from plants grown on the farm of this firm. It was sown on April 9th, 1910.

The table gives at a glance the method of manuring and the results obtained.

The formula for Potato Mixture is

Superphosphate (26% phosphates)	14 parts
Sulphate of Ammonia (20% Nitrogen)	6½ parts
Bone Flour { 1½% Ammonia, 60% Total Phosphates; 50% Phosphates Citric Sol.; 38% Lime }	¾ part
Potass. Sulphate (48% potash)	2 parts

The Kainit used contained 10% potash, the Nitrate of Soda 15% Nitrogen, the basic slag 24% of phosphate 80% Sol. in Citric acid solution.

Four pounds of leaves were gathered from each plot at the end of September, 1910, in the middle of June, 1911, and at the end of September, 1911, and carefully dried.

The percentage of moisture was estimated for each four pounds.

The percentage of moisture contained in each 44 lbs. taken together was as follows:

1910	1911 June	1911 September
86%	87%	85%

The maximum percentage of moisture was in each case found in Plot A, no manure (shade).

1910	1911 June	1911 September
90.6%	88.7%	88%

The minimum percentage of moisture was found in Plot H, except in June, 1911, when Plot J contained one per cent. less moisture than Plot H as follows:

1910	1911 June	1911 September
H 83.6%	H 85%	H 83.4%
	J 84%	

The percentages of moisture are considered to be of some slight importance, as they enable those sufficiently interested to calculate approximately the percentage of total alkaloid in the fresh leaf, and also because of the effect of shade in reducing the yield of dry material. No great stress is laid on this, however, as the moisture varies within narrow limits according to the weather prevailing when the leaves are gathered.

It is to be much regretted that for some reason not definitely ascertained a large proportion of plants on the plots have died this year, and this will render them of little value for examination, but it is hoped that new seed will be sown next year so that the work may be continued, and may possibly be extended on a larger scale on the farm.

The following was the method employed for alkaloidal estima-

tion. It was found to give concordant results, and when the final solution was allowed to evaporate spontaneously a light crystalline residue was obtained in every case.

Twenty grams of the leaf in 100 powder was exhausted in Dunstan & Short's continuous extraction apparatus with industrial methylated spirit 66 o. p., eight columns being allowed to pass. The first two columns were percolated cold and reserved to avoid subjecting the bulk of the alkaloid to prolonged heating. After exhaustion the percolates were bulked, the alcohol recovered and the soft extract obtained was estimated by the method directed in the British Pharmaceutical Formulary, 1901, of the British Pharmaceutical Conference for the estimation of total alkaloids in Ext. Belladonnæ Folii Alcoholicum, using the whole of the extract obtained. The result multiplied by five gave the percentage of total alkaloid obtained from the leaf.

Plot	Kind of Manure Cwts. per Acre	1910 (Sept.) Yield of Green Plant per Acre	1911 (June) Yield of Green Plant per Acre	1911 (Sept.) Yield of Green Plant per Acre	1910 (Sept.) Total Alkal. in dry leaf	1911 (June) Total Alkal. in dry leaf	1911 (Sept.) Total Alkal. in dry leaf	Rainfall
A	No Manure, Shade	4 tons	6¼ tons	2¼ tons	0.16%	0.255%	0.33%	1910 April 2.25 May 2.28 June 1.66 July 1.72 Aug. 1.81 Sept. .99 1911 Apr. 1.25 May 2.49 June 2.09 July .37 Aug. 1.51 Sept. .94
B	Kainit 3	8¾ tons	16¾ tons	6½ tons	0.21%	0.285%	0.472%	
C	Superphosphate 5	8¼ tons	12½ tons	7⅜ tons	0.22%	0.265%	0.60%	
D	Superphosphate 5 Kainit 3	12¼ tons	17 tons	10½ tons	0.18%	0.33%	0.64%	
E	Sod. Nitrate 1 Kainit 3	15¾ tons	26 tons	13⅛ tons	0.22%	0.415%	0.737%	
F	Sod. Nitrate 1 Superphosphate 5 Kainit 3	14 tons	29¼ tons	12⅜ tons	0.32%	0.605%	0.72%	
G	Sod. Nitrate 1 Superphosphate 5	10⅛ tons	17¼ tons	12⅘ tons	0.47%	0.46%	0.61%	
H	Sod. Nitrate 1	8¾ tons	18½ tons	6¾ tons	0.36%	0.46%	0.767%	
I	No Manure, Sun	7½ tons	18¼ tons	8⅒ tons	0.44%	0.65%	1.035%	
J	Potato Mixture 10	8¾ tons	16½ tons	9⅒ tons	0.32%	0.792%	1.02%	
K	Basic Slag 7	17¾ tons	31¼ tons	12⅖ tons	0.28%	0.47%	0.747%	

In the table it is not without significance that the highest alkaloidal result was obtained from the plot which had not been supplied with any manure, but which was fully exposed to the sun (Plot I, Sept., 1911). We believe that this is about the highest percentage of alkaloid ever recorded as having been obtained from dried Belladonna leaf. The increased yield of alkaloid in the leaves gathered from the second growth in the autumn of 1911 is of special interest.

Although we have not obtained sufficient evidence that the percentage of alkaloid in the dried leaf is materially altered by artificial manures, it would appear that in several cases the yield of green plant per acre has been largely increased.

It would also appear that in the case of Belladonna it is useless to hope that the drug shall possess anything approaching uniformity in medicinal potency, even when carefully collected and dried.

It should be mentioned that most of the work in the earlier stages of this investigation was carried out by Mr. P. E. F. Pérrèdes, B.Sc., F.L.S., to whom we wish to express our acknowledgment.

From the Laboratory of Powers-Weightman-Rosengarten Co.

THE DETERMINATION OF CALCIUM SULPHIDE

By J. Rosin
Philadelphia, Pa.

The present Pharmacopœia has no method for the actual determination of the calcium sulphide contents of calx sulphurata. The test it gives for ascertaining the presence of at least 55 per cent. of calcium sulphide has serious drawbacks tending to show a lower content of calcium sulphide than actually present. A number of samples examined in our laboratory which were known to contain more than 55 per cent would not show this percentage by the U. S. P. assay. In order to investigate this discrepancy a series of experiments were conducted as follows: Weighed quantities of calcium sulphide were mixed in glass stoppered graduated flasks with measured amounts of a standard copper sulphate solution, ten per cent hydrochloric acid added little by little, shaking vigorously after each addition, then digested on the water bath for 15 minutes, cooled, filled to the mark with water, and thoroughly mixed, filtered through a dry filter (rejecting the first 15-20 cc.) and the excess of copper determined in an aliquot portion of the filtrate. The results were always low and disagreeing widely, ranging from about 94 to 50 per cent of the calcium sulphide present. The higher results were usually obtained when more acid was used than directed by the U. S. P. Since, however, no hydrogen sulphide was observed to have escaped in any of these experiments, it was concluded that some calcium sulphide must remain occluded in the copper sulphate and was therefore not acted upon. In fact, when filtering off the excess of copper sulphate and warming the copper sulphide with diluted hydrochloric acid, hydrogen sulphide was evolved. By using strong hydrochloric acid (5-10 cc. for one gram of calcium sulphide) such low results as when diluted hydrochloric acid was used were not obtained, still they

were several per cent too low and far from condorant. This is probably due to the fact that the ordinary calcium sulphide usually contains some calcium sulphite. When the hydrochloric acid is added at least some of the sulphur dioxide liberated reacts with the hydrogen sulphide forming sulphur and water, according to the equation:

$$_2H_2S+SO_2=2H_2O+2S$$

and consequently some hydrogen sulphide is lost, the amount thus oxidized depending on the strength of the acid and the amount added at one time, the stronger the acid and the more of it is added at one time the greater will be the loss of hydrogen sulphide.

Another cause for low results is the oxidation of the copper sulphide. It is a well-known fact that copper sulphide oxidizes rather quickly in the air. While the solution with the precipitated copper sulphide is digested on the water-bath, some of the copper sulphide is oxidized, passes into the filtrate and therefore shows less copper combined with the hydrogen sulphide and consequently less calcium sulphide.

The iodine method of course cannot be applied as the sulphite will interfere.

The method outlined below was reached by the following considerations. If it were possible to bring the calcium sulphide into solution without the aid of acid, the sulphide could be precipitated by means of a metallic salt whose sulphide is insoluble in water. Further, if the sulphide of this metal be soluble in hydrochloric acid the sulphur contents of the sulphide could be determined by means of standard iodine. The problem to be solved, therefore, was how the calcium sulphide could be brought into solution without the aid of acids? The law is that when two solutions containing constituents capable of forming an undissociated compound are brought together, the undissociated body will be formed. Upon the addition of water to calcium sulphide some of it goes into solution and is dissociated into — Ca^{++} and S^{--} then —

$$S+H_2O=SH^-+OH^-$$

If then a solution of a substance which contains a constituent capable of forming an undissociated body with either the OH or SH be added, that substance will be formed. Therefore, the small amounts of calcium sulphide in solution and entering into the reaction, forming an undissociated substance, allows further calcium sulphide to become soluble and this process is thus continued until all the calcium sulphide is converted.

Now, ammonium hydroxide is but a slightly dissociated substance. In aqueous solutions ammonium salts are dissociated into NH_4^+ and X^- (where X is the acid radical). If then a solution of an ammonium salt be added to calcium sulphide, the slightly dissociated ammonium hydroxide should result and the calcium sulphide thus becomes soluble. In fact, upon addition of a solution of ammonium chloride (or sulphate) to calcium sulphide, the latter rapidly dissolves.

That the formation of the undissociated ammonium hydroxide accounts for the solubility of calcium sulphide in solutions of ammonium salts is supported by the fact that the presence of an undissociated body, such as ammonium hydroxide, greatly retards the solubility. This is in accordance with the "Law of Mass Action."

The Determination: Place about 0.2 gr. of the calcium sulphide in a 250 cc. glass stoppered flask, add 50 cc. of water, mix, introduce quickly about 30 cc. of a ten per cent solution of ammonium chloride and immediately stopper the flask. Rotate the contents of the flask for a minute or so, add quickly 15-20 cc. of a ten per cent solution of cadmium chloride and immediately insert the stopper. Shake well for a minute or two, rinse off any cadmium sulphide on the stopper into the flask, add 3-5 cc. acetic acid (30%) and heat on the water bath for 10-15 minutes. Decant through a filter, agitate the cadmium sulphide with a few cc. of acetic acid and a little water, filter and wash the precipitate with water containing a little acetic acid. Throw filter and precipitate into the precipitating flask, add 50 cc. 1/10 normal iodine and 15-20 cc. hydrochloric acid (1:1), stopper the flask, shake vigorously for a few minutes and titrate the excess of iodine with standard thiosulphate.

In order to avoid loss of hydrogen sulphide while introducing

the cadmium solution, it is best to pour some of it around the lip of the flask and the stopper open as little as possible, and add the rest of the solution. Since the precipitate is thrown back into the precipitating flask it is not necessary to bring it completely on the filter. The object of the acetic acid is to decompose sulphite. As the U. S. P. calcium sulphide usually contains but a few per cent sulphite, the addition of acetic acid may be omitted. The little sulphite that is present will readily go into solution in the amount of water used. If preferable, an ammoniacal solution of a cadmium salt can be used. In this case enough acetic acid should be added to make distinctly acid. The method was tried on three samples of calx sulphurata. By the U. S. P. test all of them showed less than 55% CaS. When tested by the iodine method sample No. 1 gave 59.0%, No. 2 60.3%, No. 3 63.5%, as calcium sulphide. By the method described the following results were obtained.

	Sample No. 1 % CaS	Sample No. 2 % CaS	Sample No. 3 % CaS
1.	56.5	57.3	58.7
2.	56.3	57.5	58.7
3.	56.5	57.5	58.9
4.	56.6	57.6	58.6
5.	56.4	57.4	

10 to 40 per cent of calcium sulphite were added to the samples tested above, and the determination as below, calculated on the weight of the original sample, gave following results:

Sample No.	% Calc. Sulphite added	% Calc. Sulphide found
1.	10	56.5
1.	25	56.8
2.	15	57.6
2.	30	57.5
3.	40	58.6
3.	40	58.9
3.	40	58.8

While the method gives results varying within a few tenths of a per cent, yet in lack of a better one it may be considered satisfactory for all practical purposes.

It may be noted here that this method is also applicable to barium sulphide.

QUININE ALKALOID AND SOME OF ITS COMPOUNDS

By George L. Schaefer
Brooklyn, N. Y.

Quinine is known in pharmacy and medicine for nearly a century as one of the most important alkaloids. Quite a number of papers have been published about it, and comparing these publications we find that there are many discrepancies in regard to the chemical and physical properties of this alkaloid. Some of these discrepancies are due to the fact that quinine salts, from which the alkaloid is made, were not produced of such a high purity years ago as they are at present. Therefore, results were obtained which cannot be considered to be quite correct for the product of today. The purpose of this paper is to correct some of the statements occurring in older publications and to call attention to some properties of quinine alkaloid of which there is nothing or little known in literature and which may be of interest to pharmaceutical chemists.

Quinine Alkaloid exists in four different forms:

A. Anhydrous.
B. With water of crystallization.
C. With hydrocarbons.
D. With hydrocarbons and water of crystallization.

GROUP A. QUININE ALKALOID ANHYDROUS

The simplest way to prepare this form of the alkaloid is to expel the water of crystallization from the official quinine hydrate by heat. Two different forms of anhydrous quinine can be obtained by this method, according to the temperature to which the hydrate is subjected.

(*a*) U. S. P. quinine hydrate, freshly prepared, containing 3 molecules or about 14.4% of water of crystallization, quickly heated in a beaker or on a watch glass, softens at a temperature

of 53-54° C. and solidifies again to a hard, lumpy mass. This product consists of quinine alkaloid still containing about 2 molecules or 10% of water of crystallization, 1 molecule being thus expelled. These hard pieces lose the rest of their water of crystallization very slowly, and a high temperature is required to get the alkaloid anhydrous. Most authors claim a temperature of 125° C. The melting point of this heavy and lumpy form of anhydrous alkaloid is 173°-174° C. If the melting point of quinine alkaloid U. S. P. is determined in the usual way, the same conditions prevail. The quinine hydrate will soften in the tube at 53°-54° C., solidifying again to a hard mass at a higher temperature, melting completely to a clear liquid at 173°-174° C.

(*b*) When, however, it is slowly and carefully dried, hydrate of quinine becomes perfectly anhydrous at a very low temperature. If exposed to warm air, the temperature of which is gradually raised up to 45°-50° C. and kept at this degree for about 3 hours and the temperature then raised to 60° C. for some time, the alkaloid gives off all its water of crystallization, becoming anhydrous. The product so obtained does not change its original appearance during the drying process and remains as a white, fluffy powder, melting completely at 171°-172° C. When this powder is exposed to a higher temperature, after being dried to constant weight at 60° C., it gives off about ½% of moisture, which it takes up again when allowed to cool in the open air. If heated to a temperature over 100° C. the white color changes slowly to yellow and brown, the quinine being partly decomposed.

It is seen that if softening of the quinine hydrate is avoided its water of crystallization can entirely be expelled at a low temperature and a perfectly white and fluffy anhydrous powder can be obtained, the melting point of which is 1°-2° lower than that of the product previously softened, or if the determination is carried out in the melting tube in the regular way, using freshly made quinine hydrate U. S. P. with the full amount of water of crystallization.

Anhydrous quinine alkaloid is also formed under various other conditions.

When precipitated from a boiling solution of a quinine salt in water, by means of an excess of ammonia water, caustic potash

or carbonate of soda, a milky solution is obtained, from which soon a part of the quinine alkaloid is separated as a soft mass, hardening quickly to hard lumps, which when powdered and dried in a cool place form a white amorphous powder of anhydrous quinine alkaloid, melting at 173°-174° C.

Another part of the quinine remains dissolved in the hot alkaline solution and will, after cooling, separate out in long silky needles of anhydrous alkaloid quinine. A much larger output of these crystals can be obtained if 3 parts of quinine hydrate are dissolved in 6 parts of hot alcohol and this hot solution quickly mixed with about 100 parts of boiling water. A part of the quinine separates out as anhydrous lumps, whereas from the remaining milky solution it is obtained as white silky needles of anhydrous alkaloid. The presence of more alcohol in the solution diminishes the output of crystals and only an amorphous thick liquid separates, which solidifies to lumps when washed with cold water. When still more alcohol is present the alkaloid is kept in solution, with no tendency to crystallize.

To produce anhydrous crystals of quinine alkaloid, aceton or methyl alcohol may be used instead of alcohol, with good results.

Dissolved in hot petroleum or petroleum benzin, hydrate of quinine separates out the water of crystallization which it contained. This being removed and the liquid allowed to cool, the whole solution will soon solidify to a gelatinous, jelly-like mass of anhydrous quinine and no crystals are formed. If this solid solution of quinine in benzin is exposed to dry air for spontaneous evaporation, amorphous anhydrous quinine will remain.

A hot, strong solution of quinine hydrate in aceton also gelatinizes after cooling. When standing for a few days this gelatinous mass is slowly transformed into beautiful, white, silky needle-shaped crystals of anhydrous alkaloid quinine.

If quinine hydrate with 3 molecules of water of crystallization is put in a flask and aceton added to it, so that the quinine is completely moistened and covered with a layer of aceton, the quinine hydrate at once forms a gelatinous sticky mass, which, after standing a few days, is entirely transformed into small needle-shaped crystals of anhydrous quinine.

When hydrate of quinine is dissolved in hot benzol to form a saturated solution and this solution shaken with dry powdered sodium carbonate until clear, to remove some of the water which is separated out, white silky needles will crystallize out after cooling, consisting of 1 molecule of quinine and 1 molecule of benzol.

From these crystals anhydrous quinine can be produced in silky needles by exposing them to a temperature of about 75° C. whereby the benzol is expelled. The melting point of this product is 171°-172°C.

Anhydrous quinine differs from the hydrate by its melting point, not softening at 53°-54° C. and its lesser solubility in ether. It also differs by the fact that its saturated solution in hot water-free benzol and many other solvents, forms a gelatinous mass, while the solutions of the hydrate either crystallize or remain liquid. The solubility in ether of the anhydrous quinine is 1:62. If the quinine is moistened before adding the ether, the solubility is again increased, this solution being very liable to gelatinize. Anhydrous quinine is also less soluble in benzol, toluol, aceton and water.

Hydrate of quinine U. S. P., when dissolved in water acidulated with sulphuric acid and then precipitated with ammonia water, is obtained again as the same product, containing 3 molecules of water of crystallization and being easily soluble in ether, also softening in the melting tube at 53°-54° C. If the same hydrate of quinine, however, is subjected to a temperature of 125° and the anhydrous alkaloid so obtained dissolved in the diluted sulphuric acid and precipitated by ammonia water, no hydrate quinine U. S. P. results, but a heavy granular product, which contains about 10% or 2 molecules of water of crystallization, being less soluble in ether. It also does not soften at a temperature of 53°-54 C.

GROUP B. QUININE ALKALOID WITH WATER OF CRYSTALLIZATION

Quinine hydrate as it usually appears in the market and as used in medicine forms a white amorphous or microcrystalline powder. The U. S. P. requires a product with 3 molecules of water of crystallization. Such a preparation is obtained when a cold diluted solution of a quinine salt is precipitated by ammonia

water or hydrate of soda and the precipitate allowed to be in contact with the mother liquor for a few hours, when the originally amorphous precipitate changes its form to very minute crystals. When carbonate of soda is used for the precipitation of the alkaloid, the precipitate remains of a more amorphous appearance.

Besides this official hydrate of quinine there exist others with 1 and 2 molecules of water of crystallization.

No stable hydrate of quinine containing more than 3 molecules of water of crystallization can be produced.

The official U. S. P. quinine hydrate with 3 molecules of water of crystallization is also not very stable and gradually dries out, even if kept in closed containers, changing its properties as to solubility in ether and melting point. When freshly made it is soluble in a little more than its own weight of ether and softens in the melting tube, without melting completely, at a temperature of 53°-54° C., hardening again and melting to a clear liquid at 173°-174° C. It also softens under water at a temperature of 45°-50° C. When such a preparation is kept for some time, it begins to lose water, becoming gradually less soluble in ether and also will not soften in the melting tube at 53°-54° C. or under water. A preparation having lost about 5% of water is soluble in about 40 parts of ether, and when 10% of water are lost it requires for solution about 60 parts, and when entirely anhydrous 62 parts of that solvent.

Hydrate of quinine containing 3 molecules of water of crystallization is obtained in long silky needles by spontane ous evapration from a solution in diluted aceton or diluted methyl-alcohol.

No crystals are obtained from a solution of the alkaloid in alcohol, chloroform or ether by spontaneous evaporation, but an amorphous mass will remain.

From a solution of the alkaloid in water of 80° C. it will crystallize in distinct needles containing 1 molecule of water of crystallization.

If quinine alkaloid is precipitated from aqueous solutions of quinine salts at different temperatures, its amount of crystal-water is diminished as the temperature is increased, whereby, however, the alkaloid can only very difficultly be obtained with

water of crystallization in exact molecular proportions, being mostly mixtures of two different hydrates. The same products are obtained when hydrate of quinine is melted under water at various temperatures.

When precipitated from a solution of a quinine salt by ammonia at a temperature of 15°-20° C. the hydrate of quinine will contain 3 molecules or about 14.4% of water of crystallization as mentioned before. This product is obtained as a very light and bulky precipitate, forming when dry a microcrystalline powder, easily soluble in ether, softening at 53°-54° C. in the melting tube and under water at 45°-50°C., while when precipitated at a higher temperature it forms soft lumpy masses, becoming hard after some time and forming a heavy amorphous product, when reduced to a dry powder, which is less soluble in ether and does not soften in the melting tube at 53°-54° C. or under water.

The following list indicates the various results obtained from solutions at different higher temperatures:

Temperature of the Solution of the Quinine Salt	Water Contained in the Alkaloid Air Dry	Solubility in Ether
30° C	12¼%	26 pts.
40° C	11%	36 pts.
50° C	10%	40 pts.
60° C	8%	47 pts.
70° C	6½%	53 pts.
80° C	5%	60 pts.
90° C	4½%	60 pts.
100° C	½%	62 pts.

The ether used is of the U. S. P. strength at 25° C. If to this ether a few drops of alcohol are added, the solubility of the above products is very much increased. The same is the case if a little water is added instead of the alcohol, though the solubility is not increased in such a marked degree by water as it is by alcohol. The addition of a small amount of alcohol to the ether also prevents gelatination of the ethereal solution of the quinine.

Anhydrous quinine alkaloid requiring 62 parts of ether U. S. P. strength is easily soluble in 3 parts of ether of a specific gravity 0,750. In absolute ether this alkaloid is soluble 1:76.

When determining the solubility of quinine alkaloid in U. S. P. ether, it very often happens that the quinine separates out as a gelatinous amorphous mass, before all the alkaloid has gone in solution. This amorphous quinine alkaloid requires for resolution a much larger quantity of the solvent as the original hydrate of quinine would require for solution and therefore no correct figures for the solubility of the latter are obtained. This is especially the case with most of the products as they are in the market, having lost 2-5% of water of crystallization. For the determination of the solubility of alkaloid quinine therefore no ether of U. S. P. strength ought to be used, but an ether of a specific gravity of 0,750, which will dissolve alkaloid quinine to a clear liquid in the proportion of 1 part of hydrate of quinine in 1½ parts of ether and 1 part of the anhydrous alkaloid in 3 parts of this ether. The question also might be considered, if it would not be better to adopt in the next edition of the U. S. P. a product which has been dried out to contain about 2% (not more) of water. In doing so the quinine alkaloid in the market, in which at present the percentage of water of crystallization may differ from 1:12%, would then be perfectly uniform.

GROUP C. QUININE ALKALOID AND HYDROCARBONS

Quinine alkaloid can be obtained from a solution in hydrocarbons in 3 different forms, according to the quantity of water present:

1. As a gelatinous, amorphous mass.
2. As crystals containing hydrocarbon.
3. As crystals containing hydrocarbon and water of crystallization.

1. Gelatinous, Amorphous Alkaloid Quinine

A hot, saturated solution of anhydrous quinine alkaloid in hot water-free petroleum, benzin, benzol, toluol, nitrobenzol, dimethylaniline and many other solvents will, after cooling and standing for a few hours, gelatinize to a clear, jelly-like mass, which, in a well-stoppered flask, can be kept for a long time with-

out showing any alteration. If not perfectly free of water a few crystals may appear after some time. If subjected to spontaneous evaporation in dry warm air the solutions in volatile solvents will leave dry amorphous quinine. When a little alcohol is added the gelatinized solutions will become liquid.

2. Alkaloid Quinine Crystallized Containing Hydrocarbons

If a hot solution of quinine hydrate in benzol 1:6 is shaken with a little powdered dry carbonate of soda until clear, the liquid filtered off and allowed to cool, there will be at first observed the formation of a few large rhombic prisms, consisting of quinine alkaloid containing benzol and water of crystallization, as described later.

When the traces of water contained in the benzol solution are taken up by these crystals, another formation of crystals appears in the shape of long silky needles. After 24 hours these crystals are separated from the mother liquor and carefully dried in the air. They consist of 1 molecule of quinine alkaloid and 1 molecule of benzol. There is a great number of hydrocarbons with which quinine forms similar compounds and in which the solvent takes the place of water of crystallization, such as toluol, xylol, aniline, orthochloraniline, and others.

GROUP D. ALKALOID QUININE WITH HYDROCARBONS AND WATER OF CRYSTALLIZATION

Quite a number of quinine compounds belong to this group, combined with various hydrocarbons of the aliphatic and aromatic series. To produce them, it is necessary that a certain amount of water is soluble in the hydrocarbon, which is used as a solvent of the quinine. Most of the hydrocarbons will not take up enough water and in this case it is necessary to add some liquid to it, by which the solubility for water is increased. Such liquids are alcohol, methylalcohol, aceton and others. With aceton I had the best results. The method generally employed to produce these compounds is as follows (below). These proportions, however, can be changed according to the nature of the hydrocarbon.

To a mixure of 1 part of aceton and 2 parts of the hydrocarbon

in a flask add water in such a quantity as the mixture may dissolve, so as to remain clear or slightly milky, heat in a water bath and add quinine alkaloid about 1 part to make a concentrated solution. Filter through cotton and set aside for crystallization for a few days. After that time the crystals can be separated and dried in a cool place. Compounds of this group may also be obtained from the gelatinous, jelly-like solution of anhydrous quinine in water-free benzol, toluol, nitrobenzol, dimethylaniline and other solvents as previously described. If to these gelatinous cold solutions an excess of water is added, the mixture stirred and set aside for a few days in a wide mouth flask, the whole amorphous mass will slowly liquefy to a thin clear liquid under simultaneous formation of large rhombic crystals.

The compounds thus obtained, as a rule, consist of 2 molecules of quinine, 1 molecule of hydrocarbon and 2 molecules of water of crystallization. They are soluble in ether, alcohol, chloroform, benzol and almost insoluble in water. Those compounds containing hydrocarbons of a high boiling point are stable at ordinary temperature, while those containing such of a low boiling point gradually lose most of their hydrocarbons, without changing their crystalline form. When heated, the volatile hydrocarbons and water of crystallization will be expelled and anhydrous quinine alkaloid remains. Its melting point is 173 - 174° C.

Some of these preparations are highly inflammable, especially those containing benzol, toluol, terebene, benzine and others. When lighted, the crystals burn, leaving a white crystalline mass of anhydrous quinine alkaloid.

When dissolved in diluted sulphuric or hydrochloric acid, the hydrocarbon separates out. An exception is made by phenols, which have the property not only to combine with quinine alkaloid, but also with neutral and acid quinine salts, with which they form well crystallized and soluble compounds. Therefore, the solution of the phenol alkaloids in diluted acid remains clear, without separating the phenols.

There exist a number of substances, which, by the above given method or in other similar ways, can be combined with quinine alkaloid. Many of the indifferent hydrocarbons will form com-

binations of this group with quinine, either from a direct solution or when the hydrocarbon is first dissolved in aceton or another suitable solvent in the presence of water. As a rule these hydrocarbons have to be little or not soluble in water.

Of the group of compounds consisting of 2 molecules quinine, 1 molecule hydrocarbon and 2 molecules of water of crystallization, the following combinations have so far been made. This list can be increased, using other solvents under similar conditions.

Benzine
Gasoline
Rhigolene
Amyl acetate
Ethyl acetate
Acetylen tetrachloride
Carbon bisulphuret
Benzol
Toluol
Xylol
Dimethylanilin
Nitrobenzol
Paranitro-chlorobenzol
Aceto-toluidin
Ortho-chloranilin

Ethereal Oils

01. Bergamottae
01. Chenopodii
01. Carvi
01. Citri
01. Cajeputi
01. Eucalypti
01. Foeniculi
01. Juniperi
01. Lavendulae
01. Menthae crispae
01. Pini
01. Rosmarini
01. Phellandri
01. Rutae
01. Sabinae
01. Succini
01. Terebinthinae
Tereben
Pinen
Terpineol
Terpinol

SOLUBILITY AND DISTRIBUTION COEFFICIENTS OF THYMOL

By Atherton Seidell, Ph.D.

Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service, Washington, D. C.

In connection with studies which are now being made in this laboratory upon thymol, a drug which is used very extensively in the treatment of hook-worm disease, it was thought that solubility data might assist in an understanding of the manner in which thymol exerts its action in the organism. The object of the distribution studies in the different oils was to ascertain if there was sufficient uniformity to permit the selection of one oil as the representative of all for future distribution studies upon other vermifuges.

The solubility and distribution determinations were made possible only by a successful method recently developed by me[1] for the quantitative estimation of thymol. According to this method the thymol in neutral solution is treated with an excess of bromine vapor and the resulting hydrobromic acid which is formed is determined by an iodometric titration after first removing the excess of free bromine by potassium iodide and thiosulphate. Since all substances which are acted upon by bromine naturally hinder the determination, advantage was taken of the volatility of thymol with steam, to separate it from its solution in oil or other interfering substances. In the steam distillation three distillates of about 150 cc. each were collected in every case and each titrated separately, thus assuring the complete removal of the thymol. It was found that the samples of oil solutions of thymol should be dropped into the distilling flask from a small weight buret, rather than introduced together with the weighing bottle or other receptacle. The explanation of this precaution is that the oil forms globules on the inner walls of the narrow weighing bottle and these globules may not be dislodged by the current of steam.

[1]Seidell, Am. Chem. Jour., *47*, 520, 1912.

The following results were obtained for the solubility of thymol in water, aqueous hydrochloric acid solutions and in several oils. A gas heated water bath maintained at constant temperature by means of a toluene regulator was used. The solutions were gently agitated by slow rotation for periods of time insuring the attainment of equilibrium.

Solubility in Water		Solubility in Aq. HCl Solutions		
t°	Gms. Thymol per 100 gms. Sat. Solution	Concentration of HCl	Gms. Thymol per 100 cc. Sat. Solution at 25°	Gms. Thymol per 100 cc. Sat. Solution at 37.2°
10	0.067	0.0	0.0995	0.132
15	0.077	0.1 normal	0.0968	0.129
20	0.088	0.5 normal	0.0884	0.121
25	0.0995	1.0 normal	0.0802	0.112
30	0.112			
35	0.126	2.5 normal	0.0612	0.0935
37	0.132	5.0 normal	0.0445	0.0772
40	0.141			

Solubility of Thymol in Several Oils

	In Olive Oil		In Cod Liver Oil		In Peanut Oil		In Linseed Oil	
t°	Sp.Gr. of Sat. Sol.	Gms. Thymol per 100 Gms. Oil	Sp.Gr. of Sat. Sol.	Gms. Thymol per 100 Gms. Oil	Sp.Gr. of Sat. Sol.	Gms. Thymol per 100 Gms. Oil	Sp.Gr. of Sat. Sol.	Gms. Thymol per 100 Gms. Oil
10	0.9376	46.2	0.9480	50.0	0.9470	73.0	0.948	62.3
15	0.9370	50.1	0.9450	52.0	0.9440	73.8	0.948	63.1
20	0.9368	56.2	0.9435	55.5	0.9417	74.6	0.948	65.1
25	0.9373	66.9	0.9430	63.1	0.9399	76.4	0.948	69.0
30	0.9389	84.5	0.9435	77.0	0.9392	83.2	0.948	78.3
35	0.9418	111.0	0.9450	102.0	0.9410	106.7	0.948	100.0
37	0.9435	124.3	0.9460	116.5	0.9425	130.5	0.948	116.5
40	0.9463	151.9	0.9478	150.0	0.9450	212.5	0.948	152.0

SOLUBILITY OF THYMOL IN SEVERAL OILS

t°	In Castor Oil		In Cotton Seed Oil		In Liquid Petrolatum	
	Sp. Gr. of Sat. Sol.	Gms. Thymol per 100 Gms. Oil	Sp. Gr. of Sat. Sol.	Gms. Thymol per 100 Gms. Oil	Sp. Gr. of Sat. Sol.	Gms. Thymol per 100 Gms. Oil
10	0.935	81.2	0.942	56.2	0.8795	3.1
15	0.942	90.2	0.943	64.0	0.8798	3.95
20	0.950	101.5	0.944	74.2	0.8802	5.6
25	0.958	116.5	0.945	89.4	0.8806	9.78
30	0.967	137.0	0.946	113.7	0.8824	16.3
35	0.975	165.0	0.948	146.5	0.8860	25.5
37	0.979	180.0	0.9485	166.5	0.8884	29.9
40	0.984	213.0	0.949	217.5	0.8944	38.9

The Distribution of Thymol between Water and Oils. In all of these experiments weighed quantities of thymol were added to accurately measured amounts of water and oil, usually 10 cc. of oil and 100 cc. water, and the mixtures rotated at the stated temperatures for two days or longer. After allowing enough time for the liquid layers to separate as completely as possible, the lower aqueous layer was withdrawn and its contained thymol determined by the above-mentioned titration method. The amount of thymol in the oil layer was then calculated by difference and the concentration of the thymol per unit volume of the oil layer was estimated with the aid of the specific gravity read from curves. These latter were plotted from the determinations of the specific gravity of known mixtures of the oil and thymol. This plan was found necessary since the concentration in the oil layer was so great that very different values are obtained for concentrations per unit volume of solvent and unit volume of the solution. The following results were obtained for olive, cod liver and peanut oils.

DISTRIBUTION OF THYMOL BETWEEN WATER AND OLIVE OIL

Results at 37.2°

Gms. Thymol per 100 cc.		C_o
Aqueous Layer (C_w)	Olive Oil Layer (C_o)	C_w
0.1087	46.35	427
0.1053	43.60	414
0.0987	40.67	412
0.0921	36.67	398
0.0807	33.48	415
0.0695	28.00	403
0.0682	27.93	410
0.0395	16.31	403
0.0381	16.24	426
0.0215	8.83	410
0.0122	4.64	382
0.0122	4.61	378
Average		406

Ratio of the Solubilities $\frac{52.3}{0.132} = 397$

Results at 25°

Gms. Thymol per 100 cc.		C_o
Aqueous Layer (C_w)	Olive Oil Layer (C_o)	C_w
0.1014	44.95	443
0.0848	36.34	428
0.0655	29.43	450
0.0349	16.26	465
0.0185	8.80	474
0.0106	4.54	430
Average		450

Ratio of the Solubilities $\frac{37.6}{0.0995} = 377$

DISTRIBUTION OF THYMOL BETWEEN WATER AND COD LIVER OIL

Results at 37°

Gms. Thymol per 100 cc.		C_o
Aqueous Layer (C_w)	Cod Liver Oil Layer (C_o)	C_w
0.1099	43.81	399
0.0862	32.90	380
0.0574	22.51	392
0.0250	8.85	357
Average		382

Ratio of the Solubilities $\frac{53.3}{0.132} = 404$

Results at 25°

Gms. Thymol per 100 cc.		C_o
Aqueous Layer (C_w)	Cod Liver Oil Layer (C_o)	C_w
0.1079	49.00	454
0.0816	32.58	400
0.0371	16.18	436
0.0127	4.57	359[1]
Average		423

Ratio of the Solubilities $\frac{40.7}{0.0995} = 409$

[1]Omitted from average.

DISTRIBUTION OF THYMOL BETWEEN WATER AND PEANUT OIL AT 25°

Gms. Thymol per 100 cc.		$\frac{C_o}{C_w}$
Aqueous Layer (C_w)	Peanut Oil Layer (C_o)	
0.1077	46.48	431
0.0786	32.45	413
0.0395	16.16	409
0.0088 (?)	4.63	523[1]
	Average	417

Ratio of the Solubilities $\frac{36.5}{0.0995} = 367$

The above results show in each case that at 25° the distribution coefficient as determined is considerably higher than calculated from the solubilities. These higher values are probably due to an imperfect separation of the two liquid layers at this temperature.

The values for the distribution coefficients as calculated from the solubility determinations at 37° are as follows:

	Gms. Thymol per 100 cc. Sat. Solution in			$\frac{C_o}{C_w}$
Oil	Oil (C_o)	Water (C_w)		
Liquid Petrolatum	20.4	0.132		155
Cod Liver Oil	50.9	0.132		386
Linseed Oil	51.0	0.132	Average	386
Olive Oil	52.3	0.132	393	397
Peanut Oil	53.3	0.132		404
Cotton Seed Oil	59.3	0.132		449
Castor Oil	62.9	0.132		477

Although these results show that at 37° the distribution coefficient is practically the same for four of the oils and not very different for two others, an examination of the solubility results at the lower temperatures shows that such close agreement will not be obtained. Nevertheless, in studies of drugs it is their conduct at body temperature, viz., 37°, which is of particular interest and it may, therefore, be concluded that for this purpose olive oil is fairly representative and its selection for distribution studies is justifiable.

[1]Omitted from average.

CADMIUM NITRATE IN QUALITATIVE ANALYSIS

By Anton Vorisek
New York City, N. Y.

I. Employment of Cadmium to Subdivide the Anions of the Silver Nitrate Group

In an attempt to systematize the procedure for the detection of the anions of the second ($AgNO_3$) group, it was soon found that the precipitation of these ions as Ag salts at the beginning presented many difficulties and that the results of the subsequent treatment of the precipitate with solvents were not satisfactory. In the second group of anions are included Cl, Br, I, CN, SCN, $Fe^{II}(CN)_6$, $Fe^{III}(CN)_6$ and S. The S, $Fe^{II}(CN)_6$ and $Fe^{III}(CN)_6$ ions were regarded the chief disturbing factors. It appeared desirable to remove them before tests for the other members of the group are to be made. A suitable reagent which would completely precipitate them and not precipitate the other radicals was to be found. Among those tried for this purpose were the salts of Zn, Ni, Co, Mn and Cu. While Zn salts, which are being used by some and those of most of the other metals precipitate the anions in question, they also precipitate all or nearly all of the CN. It was argued that the detection of CN away from $Fe^{II}(CN)_6$ and $Fe^{III}(CN)_6$ would be a more desirable as well as a simpler operation. Cd salts did not precipitate all of the CN; this lead to the investigation of the reactions of Cd salts with some of these anions.

The Insoluble Cd Salts of the Anions of the Second Group

Ordinarily Cd salts do not precipitate from solutions of their salts four of the ions Cl, Br, I and SCN, but should precipitate S, $Fe^{II}(CN)_6$, $Fe^{III}(CN)_6$ and CN since the Cd salts of the latter ions are classed as insoluble.

Precipitation of $Fe^{III}(CN)_6$ by Cadmium Nitrate

The existence of a number of Cd ferrocyanides whose composition changes after precipitation has been shown by Miller.[1] Their

[1] J. Am. Chem. Soc. *26* 226-34.

constitution at the time of formation depends on the reaction of the medium and the reagent which is in excess. Since the object here was to completely precipitate the $Fe^{III}(CN)_6$, the Cd salt had to be used in excess. It was soon found that the reaction can be best brought about in neutral or nearly neutral solutions. The $Cd(NO_3)_2$ solution employed had a slight acid reaction (10 cc. of it required 0.1 cc. of tenth normal KOH V. S. for neutralization) and was nearly fifth normal. To neutralize the acidity of the mixture of the Cd solution and $K_4Fe(CN)_6$, the N/10 KOH solution was run in from a buret in the presence of phenolphthalein. A much larger volume of the alkali solution was consumed than the acidity of the Cd solution used called for. This was at first thought to be due to an excess of the $Cd(NO_3)_2$ which would consume KOH to form $Cd(OH)_2$. The explanation appeared satisfactory, since by increasing the amount of the Cd salt a large volume of the KOH solution was required. But later, when the least volume of the $Cd(NO_3)_2$ solution required to completely precipitate the $Fe^{II}(CN)_6$ ions was found, there still was a considerably large volume of the KOH V.S. consumed in the reaction.

THE PROPORTIONS OF THE REAGENTS

In order to find the relative amounts of the three reacting substances, namely, $K_4Fe(CN)_6$, $Cd(NO_3)_2$ and KOH, nearly fifth-normal V.S. were made of the first two, while the KOH solution used was tenth normal. The reagents were measured from burets. The results were calculated into fifth-normal values for easier comparison. In general a definite volume of the ferrocyanide solution was mixed with the $Cd(NO_3)_2$, an equal volume of H_2O was added and the mixture neutralized with the KOH solution. It was then slowly heated to boiling or placed on steam bath for 5 to 10 minutes. After filtration portions of the filtrate were tested for the absence of $Fe^{II}(CN)_6$ and of Cd ions.

The results of many experiments showed that certain minimum amounts of the Cd salt and KOH must be added in order to bring about a complete precipitation of the $Fe^{II}(CN)_6$ ions. For 10 cc. of N/5 $K_4Fe(CN)_6$ at least 6.7 cc. of N/5 $Cd(NO_3)_2$, and 2 cc. of N/5 KOH solution had to be added. With these quantities and under the conditions of the outlined treatment, a complete precipi-

tation resulted; the filtrate failed to react with $CuSO_4$ and acetic acid and gave only a light yellow color with H_2S (slight excess of Cd). A larger excess of $Cd(NO_3)_2$ caused no difficulty nor had a small amount of the alkali any effect on the precipitate in the cold; but a larger excess of it, particularly on heating, caused the appearance of $Fe^{II}(CN)_6$ ions in the filtrate.

Precipitation of $Fe(CN)_6$ by Cadmium Nitrate

A solution of $K_3Fe(CN)_6$ nearly N/5 was freshly prepared. The conditions of treatment were those outlined for the ferrocyanide. To completely precipitate the $Fe^{III}(CN)_6$ ions in 10 cc. of the N/5 solution at least 12.5 cc. of N/5 $Cd(NO_3)_2$ solution and 2.2 cc. of N/5 KOH V.S. had to be used. With these amounts the filtrate was clear and failed to give more than a very faint bluish color with dilute HCl and a $FeSO_4$ solution. It was found that a complete precipitation of the $Fe^{III}(CN)_6$ ions by means of $Cd(NO_3)_2$ is not as readily accomplished as is the precipitation of the ferrocyanide. With KOH below its limit the filtrate is mostly cloudy as it is also when a large excess of the Cd salt is added. However, any remaining $Fe^{III}(CN)_6$ ions may readily be reduced by SO_2 and removed as Cd ferrocyanide. Ammonium salts when present in large amounts also interfere.

Reaction of Cadmium Nitrate with CN Ions

When varying quantities of $Cd(NO_3)_2$ were caused to react with KCN in solution a precipitate formed, but the filtrate always contained CN ions. To determine the approximate amount of CN not precipitated a stronger solution of KCN was titrated with $AgNO_3$ V.S. (Liebig's method) and diluted with water so that the two were equal, volume for volume. Definite volumes of the KCN solution were then mixed with varying volumes of the Cd reagent and the mixture filtered either hot or cold, with or without added KOH. The precipitate was washed with about 50 cc. of water and the washings combined with the filtrate. The liquid was then made alkaline with NH_4OH, 0.1 gm. of KI added and the CN present titrated with the N/10 $AgNO_3$ V.S. In view of the fact that $Cd(CN)_2$ is considered insoluble, the results were quite surprising.

PRECIPITATION OF CN IONS BY CADMIUM NITRATE

	N/5 KCN	N/5Cd $(NO_3)_2$	Volumes in cc.		Remarks
			N/5 KOH	Filtrate consumed of N/10 $AgNO_3$	
1	10	10	none	7.5	boiling hot
2	10	20	none	8.0	warmed only
3	10	15	2.5	9.0	luke warm
4	10	12.5	2.5	9.1	50 cc. H_2O added before precipitation. Heated on stem b.
5	10	12.5	2.5	9.4	50 cc. H_2O added after pptn., pptn. washed with hot water
6	10	12.5	2.5	9.4	10 cc. of H_2O added and boiled
7	10	12.5	2.5	9.4	cold

In some cases a flocculent precipitate separated during the titration before the end point was reached. The precipitate was soluble in more NH_4OH and was evidently $Cd(OH)_2$. As 10 cc. of the KCN solution consumed 10 cc. of the $AgNO_3$ V.S. it appears that under the condition of the treatment in experiments 5, 6 and 7, 94% of the KCN is not precipitated. With mixtures of KCN and ferrocyanide, ferricyanide and also citrate and tartrate this percentage was found less, 82 to 88% being retained in solution. But the amount is considered sufficiently large to permit of the detection of CN ions in the filtrate from the Cd precipitate and apart from $Fe^{II}(CN)_6$ and $Fe^{III}(CN)_6$.

THE SULFIDE OF CADMIUM

The precipitation of S by cadmium salts was thought to be so well known that no experiments covering the reaction were carried out. With $Cd(NO_3)_2$ in excess and under the conditions of the preceding experiments the precipitation of S will be complete.

The relative amounts of reagents thus far established were determined for qualitative purposes and are to be considered as approximate.

SUBDIVISION OF THE ANIONS OF THE SECOND GROUP

Since it was shown that $Fe^{II}(CN)_6$, $Fe^{III}(CN)_6$ and S ions may be completely precipitated by $Cd(NO_3)_2$ and KOH under the conditions of treatment which is outlined and which favor the retention in solution of the CN ions, a procedure for their removal based on the results of the experiments was devised. The solution to be treated should be neutral or slightly alkaline. It is tested for the presence of these ions in three small portions of 0.5 to 1 cc. each. One portion is made alkaline with KOH, diluted with water and tested with a dilute solution of $Na_3(NO)Fe(CN)_5$. The other two are acidified with dilute HCl, diluted with water and tested for $Fe^{II}(CN)_6$ with $Fe^{III}Cl_3$, and for $Fe^{III}(CN)_6$ with pure $FeSO_4$ solution. A deep-red color with $FeCl_3$ indicates SCN. When one or all three of these anions are found present they are removed by the following procedure:

PROCEDURE

A larger volume of the solution (some 10 cc.) is neutralized, if need be, with KOH or dil. HNO_3, an excess of 10% $Cd(NO_3)_2$ solution is added and the KOH solution run in carefully until a faint alkaline reaction is produced. The excess of the alkali is then neutralized with a few drops of the Cd solution or of dilute acetic acid, the mixture diluted with water and heated on steam bath for 5 to 10 minutes. It is filtered while hot and the precipitate rejected. With $Fe^{III}(CN)_6$ ions the filtrate is apt to contain traces of them. To remove the last traces of the ferricyanide it is necessary only to add a few drops of H_2SO_3 solution, heat to boiling and filter.

Of the filtrate a small portion (1/4) is now tested for CN with FeSO + KOH, heating and then adding a drop of $FeCl_3$ and HCl to acid reaction. SCN ions interfere but may be eliminated by means of $HgCl_2$ solution. The rest of the filtrate (3/4) is acidified with dilute HNO_3 and treated for the detection of Cl, Br, and I ions in the usual manner.

SUMMARY OF THE RESULTS

Cadmium nitrate may be employed in the analysis of the second group of anions to remove S, $Fe^{II}(CN)_6$, $Fe^{III}(CN)_6$.

The minimum amounts of $Cd(NO_3)_2$ and of KOH required for a complete precipitation were approximately determined. The amount of KOH consumed is out of proportion to the acidity of the Cd solution used and evidently takes part in the formation of the precipitates.

Of the CN ions more than 4/5 remain unprecipitated under the conditions of the treatment employed.

Procedure for the subdivision of the anions of the second group is outlined.

II. PRECIPITATION OF TARTARIC AND CITRIC ACIDS BY CADMIUM

The commonly used method of detection and separation of tartaric and citric acids by means of $CaCl_2$ in the presence of NH_4Cl[1] requires much time for the deposition of the Ca tartrate and presents certain other difficulties. The NH_4Cl added to keep Ca-citrate in solution not only retards the separation of the tartrate but makes the process unsuited for small quantities of these acids, particularly the tartaric acid which is apt to be overlooked, as pointed out by Fresenius.[2] That NH_4 salts of different anions may differ in their influence on the rapidity and extent of precipitation of the Ca-tartrate was suspected and confirmed in the following experiments. In these equal volumes of the neutral tartrate solution and of the NH_4 salts solutions of nearly equivalent strength were mixed, the solutions made alkaline with NH_4OH, $Ca(NO_3)_2$ solution added in excess and, after mixing, allowed to stand.

This shows that in the presence of NH_4NO_3 and NH_4SCN the precipitation of tartaric acid by $CaCl_2$ may not be complete even after 24 hours.

The separation of oxalic acid from tartaric can not be readily brought about as the whole of tartaric acid precipitates with the CaC_2O_4 in acetic acid solutions.[3] The question now arose: Could another reagent be used?

[1]Qual. Chem. Anal. xi ed. 343.
[2]Ibid., 306
[3]Ztsch. anal. chem. *38*, 33–5.

EFFECT OF NH_4 SALTS ON THE PRECIPITATION OF CATARTRATE

	Salt added	Ppt. began at room temp. in min.	Amt. of ppt. in 1hr.	Character of ppt.	Filtrate after 24 hours tested with $Cd(NO_3)_2$
1	NH_4Cl	20	large bulky	crystal.	no ppt.
2	NH_4NO_3	45	small amorph.	amorphous	ppt.
3	$(NH_4)_2SO_4$	40	large	crystal.	no ppt.
4	$NH_4C_2H_3O_2$	35	less than 3	crystal.	no ppt.
5	NH_4SCN	30	less than 4	crystal.	ppt.

SOME OTHER REAGENTS

Barium acetate readily precipitates both acids from neutral or weakly alkaline solutions, but the salts formed do not permit of a ready separation. Pb, Hg, and Ag salts precipitate, in addition, a large number of other anions and therefore can be used only in their absence. Zn, Mn, Ni and Sn salts react with tartrates and not readily with citrates, but the precipitation of tartrate ions is not sufficiently complete. Finally, cadmium salts were tried. That $CdCl_2$ gives a jelly-like precipitate with citric acid is stated by Gooch and Browning[1] and also by Perkin,[2] but otherwise the reactions of cadmium salts with these acids appear to be little known and not used. In fact, Perkin adds[3] that "cadmium salts give no precipitate with tartrates." This statement was readily disproved as soon as a solution of $Cd(NO_3)_2$ was added to a solution of a neutral tartrate. A small precipitate formed at once and increased on mixing. However, the amount of it was out of proportion to the amounts of the reagents and indicated that the precipitation was not complete.

THE REACTION OF TARTARIC ACID WITH CADMIUM NITRATE

Since cadmium tartrate is soluble in acids neutral or nearly neutral solutions should be favorable to complete precipitation. In carrying out a large number of experiments in order to deter-

[1]Outl. anal. Chem. II ed. 1909.
[2]Qual, chem. anal. II ed. 1905.
[3]Ibid.

mine if complete precipitation can be brought about, it was found that here, as in the case of certain anions of the second group,[1] a much larger volume of N/10 KOH V.S. was consumed than corresponded to the acidity of the $Cd(NO_3)_2$ solution used. The least volumes of the standard solution of Cd $(NO_3)_2$ and of KOH required to completely precipitate a given quantity of tartaric acid were approximately determined. Cadmium nitrate being, unlike other Cd salts, strongly ionizable[2] and showing little tendency to form complex salts, was found preferable to the chloride or sulfate. A nearly N/5 solution, standardized gravimetrically, was employed. The tartrate solution was obtained by neutralizing a N/5 tartaric acid solution with KOH. The KOH V.S. was tenth normal.

OUTLINE OF THE TREATMENT

The Cd nitrate solution was, as a rule, run from a buret into a definite volume of the tartrate solution — reversing the order was without effect — the KOH solution added, the mixture shaken, heated to boiling and kept agitated while boiling one to two minutes. A portion of it was filtered while hot and tested for the absence of $C_4H_4O_6$ and Cd ions. For rapid testing for tartrates a neutral, 5% solution of $Pb(C_2H_3O_2)_2$ was used and found very serviceable, the reaction appearing with one part of tartrate in 10,000 of H_2O. For Cd a solution of H_2S was used.

THE RELATIVE AMOUNTS OF THE REAGENTS

The results, which for easier comparison were calculated into N/5 values, show that 10 cc. of N/5 tartaric acid require, for complete precipitation, at least 22 cc. of N/5 $Cd(NO_3)_2$ solution and 11 cc. of N/5 KOH V.S.

Under the conditions of the treatment just outlined and with the amounts of reagents represented the filtrate gave no test with the neutral Pb acetate and contained only traces of Cd. While more than 22 cc. of the Cd reagent had no noticeable effect on the precipitate, an excess of the alkali, especially on heating, caused the appearance of $C_4H_4O_6$ ions in the filtrate. It was here observed that a comparatively large excess of the alkali may be

[1]Vorisek, Cd in Qual. Analysis, (I).
[2]Drucker, Abegg's Hand. Anorg. Chem. II, 482.

added without the mixture becoming alkaline in reaction provided it is well boiled. Heating was found to promote precipitation and cause the somewhat flocculent precipitate to become more granular. The addition of an equal volume of water before or after the reaction ensued had no noticeable effect, although when diluted with two or more volumes of water the filtered mixture gave test for both tartaric acid and Cd. This would indicate that tartrate solutions to be precipitated by $Cd(NO_3)_2$ should not be weaker than tenth-normal.

While not the object of this investigation the probable composition of the Cd precipitate was of great interest. If in these approximate values obtained an allowance were made for the excess of each of the reagents, which it is reasonable to suppose are necessary to make, the precipitation of the tartrate complete, than the ratios of $Cd : C_4H_4O_6 : 2K = 2 : 1 : 1$ would indicate the formation of a compound of the formula: $Cd_2(OH)_2C_4H_4O_6$ according to the equation:

$$2\,Cd(NO_3)_2 + K_2C_4H_4O_6 + 2\,KOH = Cd_2(OH)_2C_4H_4O_6 + 4\,KOH$$

The equation must, however, remain tentative in character until analyses of the precipitate and other more accurate determination, which are to be undertaken, shall be secured.

PRECIPITATION OF CITRIC ACID BY CADMIUM NITRATE

A definite volume of a neutral citrate solution was treated in each experiment with $Cd(NO_3)_2$ and KOH solutions as in the case of tartaric acid. The precipitate which readily forms is amorphous, jelly-like and distinctly translucent in the cold. It becomes less dense when the alkali is added and, on boiling, assumes a granular form. To completely precipitate the citric acid in 10 cc. of its N/5 solution were required at least 11.2 cc. of N/5 $Cd(NO_3)_2$ solution and 2.2 cc. of the KOH, also N/5. Under the conditions of treatment the filtrate gave no reaction with neutral Pb acetate and contained only a minute amount of Cd. More than 11.2 cc. of the Cd reagent produced no effect, but an excess of the KOH, on heating, showed a decided solvent action on the precipitate. The addition of two volumes or less of water was without effect.

The relative amounts of the reagents thus found do not make the Cd compound of citric acid correspond to any one of the for-

mulæ for calcium citrates that it was possible to locate,[1] though it would be close to $Cd_9H_2(C_6H_5O_7)_{5x}Aq$.

The approximate amounts of reagents consumed in precipitation of the two acids by $Cd(NO_3)_2$ are:

Acid	cc. of N/5 Acid	cc. of N/5 $Cd(NO_3)_2$	cc. of N/5 KOH
Tartaric	10	22.0	11.0
Citric	10	11.2	2.2

THE INFLUENCE OF OTHER COMPOUNDS ON THE PRECIPITATION OF BOTH ACIDS

Ammonium salts were found to interfere very materially. The most efficient are the acetate and thiocyanate. No interference was observed when the NH_4 of these salts was replaced by K or Na. The presence of CN, S, $Fe^{II}(CN)_6$ and $Fe^{III}(CN)_6$ had no other influence than that more of the reagents had to be added to precipitate the Cd salts of these together with the organic ions. Moderate quantities of sugar and of glycerol caused no disturbance of the reaction.

Substitution of K, Na or NH_4 carbonates for KOH was tried without success. These reagents had to be used in much larger quantities than expected and some of the $CdCO_3$ formed often passed through the filter. Ammonium hydroxide can not be used on account of the ready solubility of the precipitate in it.

RECOVERY OF THE ACIDS FROM THE PRECIPITATE

Since it was shown that tartaric and citric acids can be completely precipitated from solutions by means of $Cd(NO_3)_2$ the recovery of these acids for the purpose of identification may be brought about. The precipitate containing the two Cd salts is washed with water, either dissolved in dilute acetic acid or suspended in water, and decomposed with H_2S. A large excess of the H_2S gas should be avoided to prevent the formation of a col-

[1]Beistein, H. O. Chem. I, 838.

loidal CdS which partly redissolves.[1] From 10 cc. portions of N/10 solutions of each acid, precipitated by $Cd(NO_3)_2$ and the precipitate treated in the manner just outlined, were recovered 93.85% of citric and 93.8% of tartaric acid. When mixed, the isolated acids are separated by means of $KC_2H_3O_2$ solution in the usual manner. Tartaric acid is best identified by warming the precipitated $KHC_4H_4O_6$ with $AgNO_3$ and a trace of NH_4OH. The addition of a drop or two of NaOH was found to assist in the production of the silver mirror. For citric acid the Denigés test[2] with $HGSO_4$ solution and $KMNO_4$ was found very sensitive and quite satisfactory.

ISOLATION AND IDENTIFICATION OF TARTARIC AND CITRIC ACIDS

The conditions under which tartaric and citric acids are precipitated are identical with those which obtain in the subdivision of the anions of the second group by means of $Cd(NO_3)_2$ solution.[3] The isolation and detection of these acids in the presence of S, $Fe^{II}(CN)_6$ and $Fe^{III}(CN)_6$ and the removal of the latter ions may therefore be taken up in one treatment. The procedure here outlined is based on the solubility of Cd citrate and tartrate in cold, dilute acetic acid in which S, $Fe^{II}(CN)_6$, $Fe^{III}(CN)_6$ C_2O_4 and SiO_4 compounds of Cd are insoluble. Interfering are NH_4 salts, PO_4 and CrO_4 ions. When found present, PO_4 ions are removed from a weakly alkaline solution containing NH_4NO_3 by means of $Ca(NO_3)_2$. For CrO_4 ions $Ba(NO_3)_2$ should be added after the addition of an acetate has been made. The ammonium salts are decomposed by boiling with KOH or NaOH. Any Ba or Ca should, of course, be removed by Na_2CO_3.

PROCEDURE

To the neutral, not too dilute solution 10% $Cd(NO_3)_2$ solution is added until the reagent is in excess, and then KOH solution is carefully run in to a slight alkaline reaction. The excess of the alkali is then neutralized with a few drops of the Cd reagent and the mixture, if dense, is diluted with water and kept on a steam bath for 5 to 10 minutes. It is filtered while hot, the precipitate

[1]Prost, Bull, Ac, Belg. *14*, 312.
[2]Deniges: Compt. rendus *130*, 32–5.
[3]Vorisek, Cd in Qual. Anal. (I).

washed with water, removed from the filter and stirred with cold, 5% acetic acid until completely disintegrated. After filtering, the solution of the cadmium citrate and tartrate is evaporated on steam bath to near dryness and the residue taken up with water. The Cd is then precipitated with H_2S and the CdS removed by filtration. The filtrate gives the acids on evaporation to dryness. In the residue the free tartaric and citric acids are then identified as has been indicated.

SUMMARY OF THE RESULTS

Ammonium acetate and thiocyanate are the most effective NH_4 salts in preventing precipitation of calcium tartrate. Tartaric and citric acids may be completely precipitated from neutral solutions by $Cd(NO_3)_2$ and KOH.

The minimum amounts of these reagents required to make the precipitation complete were approximately determined. In the case of tartaric acid the relative amounts of the reagents consumed point to $Cd_2(OH)_2C_4H_4O_6$ as the formula of the precipitate which is tentatively reported pending more accurate data to be secured.

The results with citric acid fail to indicate an agreement with any one of the formulæ extant.

Recovery of the acids from the Cd precipitate was made and their isolation and identification discussed.

A procedure for the detection of these two acids and the removal of certain ions of the second group in one treatment was devised and is outlined.

ORIGINAL COMMUNICATIONS

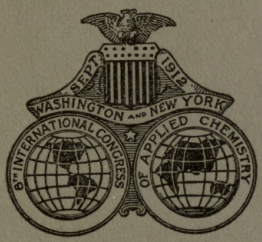
EIGHTH INTERNATIONAL

CONGRESS OF APPLIED CHEMISTRY

Washington and New York

September 4 to 13, 1912

SECTION VIIIc: BROMATOLOGY



VOL. XVIII.

ORIGINAL COMMUNICATIONS

EIGHTH INTERNATIONAL

CONGRESS OF APPLIED CHEMISTRY

Washington and New York

September 4 to 13, 1912

SECTION VIIIc: BROMATOLOGY

VOL. XVIII.

The matter contained in this volume is printed in exact accordance with the manuscript submitted, as provided for in the rules governing papers and publications.

La matière de ce volume a été imprimée strictement d'accord avec le manuscrit fourni et les règles gouvernant tous les documents et publications.

Die in diesem Heft enthaltenen Beiträge sind genau in Übereinstimmung mit den uns unterbreiteten Manuskripten gedruckt, in Gemässheit der für Beiträge und Verlagsartikel geltenden Bestimmungen.

La materia di questo volume e stampata in accordo al manoscritto presentato ed in base alle regole que governano i documenti e le publicazioni.

THE RUMFORD PRESS
CONCORD·N·H·U·S·A·

ORIGINAL COMMUNICATIONS

TO THE

EIGHTH INTERNATIONAL CONGRESS

OF

APPLIED CHEMISTRY

APPROVED

BY THE

COMMITTEE ON PAPERS AND PUBLICATIONS

IRVING W. FAY, Chairman

T. LYNTON BRIGGS
F. W. FRERICHS
A. C. LANGMUIR
JOHN C. OLSEN
JOSEPH W. RICHARDS
E. F. ROEBER
A. F. SEEKER

SECTION VIIIc. BROMATOLOGY.

Executive Committee.

President: W. D. Bigelow, Ph.D.
Vice-President: A. L. Winton, Ph.D.
Secretary: H. A. Baker, B.A.
Paul Rudnick
Chas. D. Woods, Sc.D.

Sectional Committee.

Charles S. Ash
E. H. S. Bailey, Ph.D.
H. E. Barnard, B.S.
F. W. Bedford, M.S.
Lucius P. Brown
R. E. Doolittle, B.S.
Richard Fischer, Ph.D.
Elton Fulmer, M.A.
H. S. Grindley, Sc.D.
Rudolph Hirsch, B.Sc.
M. E. Jaffa, M.S.
C. Langley
C. F. Langworthy, Ph.D.
H. M. Loomis, B.S.
William McPherson, D.Sc., Ph.D.
G. F. Mason, M.S.
L. H. Merrill, Sc.D.
Andrew S. Mitchell, Ph.C.
Harry Snyder, B.S.
John Phillips Street, M.S.
A. G. Woodman, B.S.

and the Sectional Executive Committee.

VOLUME XVIII

SECTION VIIIc: BROMATOLOGY

CONTENTS

THE RELATION OF THE CHEMIST TO THE WINE INDUSTRY

CHARLES S. ASH

San Francisco, Cal.

I think that I am correct when I state that there are few, if any, industries where it is so difficult for the chemist to prove his necessity as in the manufacture of wine. This is due to the following facts:

The most common difficulty is that people connected with the wine industry have absolutely no idea of chemical science and cannot see any use for the chemist in their business.

The wine industry is not what we would call a true manufacturing industry, as for example, the sugar industry; therefore, the chemist has no value in a mathematical capacity. He does not trace out losses in manufacture as does the sugar chemist. It is apparent to any business man that if there are 10,000 lbs. of raw sugar put into a refinery in a day and if 9,500 lbs. are recovered in the refined product that he has a loss of 5 per cent. The chemist here is of value to him to ascertain definitely the loss of manufacture, to help him minimize that loss, trace up leaks, improve processes of refining and many other incidentals, which has made the sugar chemist renowned throughout the world. This example holds good in any manufacturing process where the raw material is converted into a finished article. Even in the kindred industry of grain distillation, we have the obvious necessity for the chemist; for it is a self-evident fact to the distiller that the man, who can increase the yield of alcohol from a given weight of cereal, is of value to him. In the yeast industry (another kindred industry) this example holds good. In fact an instance has come to my knowledge where a chemist has increased the yield of yeast for his employers over 30 per cent. All of these things mean profit to the merchant and profit is "Raison d'etre" of all enterprise. In other manufacturing industries, the chemist has proved his value by making

immense fortunes in by-products. In a strictly chemical line, as in the manufacture of chemicals, as well as in the manufacture of dyes and colors, the whole industry is absolutely dependent upon chemical laws hidden or obvious. The innumerable new dyes and colorings are the product of brains alone and would never have seen the light of day but for the master minds, who have built up these synthetical products. As grapes are very poor in by-products, and as wine is a natural product, the wine chemist was shut out from usefulness in this direction.

Compared to the above industries, the wine industry is the most venerable; wine having been made by the Egyptians many centuries before Christ. It is mentioned throughout the Old Testament repeatedly. As wine has made itself spontaneously long before such a thing as chemistry was ever dreamt of (that is our conception of exact chemical science) it was considered a natural product. About fifty or sixty years ago, when Pasteur made his classical researches, considerable light was thrown on the subject and for the next fifty years, his views made themselves slowly felt throughout the world. The first effect was in the beer industry where yeast was planted in sterile medium and the quality of the beer has depended greatly upon the quality of the yeast. In the wine industry this was not the case, as the process of making wine was roughly this: The grapes were crushed and allowed to ferment. They fermented spontaneously on their own yeast and made good or bad wine as the composition of the grape, climate, temperature during the fermenting period and other conditions allowed. We hear to this day the most abused phrase of "the especially fine vintage of 188—". This was simply that the grapes ripened well and that the temperature during the fermenting period was ideal. Such a thing as controlling the temperature was unthought and unheard of.

When the chemist looked for employment in the wine industry, he was asked what he could possibly do, what he could possibly find out that an expert taster did not know. How could he benefit the wine industry in any way? He replied that he could analyze the wines, determine the amount of alcohol, total acids, volatile acids, solids, tannin, glycerol, etc. This brought the reply, "Well, if I did know, what good is it to me? I am glad

to know that wine has, for example, 12 per cent alcohol, and has a total acid of 6 parts per thousand and volatile acid of one part per thousand but, after I do know it, what does it mean to me? I am very pleased to know it but it does not do me any good. It does not tell me whether the wine is good, bad or indifferent; I can tell from my taste all those things. There is nothing I cannot tell. I have been running this wine business for the last fifty years without a chemist and made money, and I should be able to run it a great many more years without one."/ What he said was, in a way, absolutely true. This was the unfortunate thing because a chemist, applying for a position in the wine industry, is not a wine man and knows nothing about the nature of wine. His value only starts to be of importance when he does become a wine man. The fallacy of the wine merchant at this time (fifteen or so years ago) was that he did depend on the chemist, that, in fact, all over the world preservatives were used; a chemical product itself, preservative values having been found by chemists. Therefore, no matter how troublesome a wine may have been, preservatives in a great or small quantity were added, and the wine kept indefinitely. Chemists, however, were finally employed in the wine industry. I think that the first wine laboratory of any corporation in the United States, and possibly one of the first in the world, was formed in 1895; Before long the chemist was able to tell the merchant what 12 per cent alcohol meant, what the total acidity of .5 per cent meant and what 2½ per cent of solids meant; and, furthermore, what the expert taster could taste, what he could not taste and could also prove the taster was correct or incorrect in his opinion of a wine. For example, if he considered a wine sound that had .150 grams volatile acid (as acetic) per 100 c.c.; then the taster was wrong, that the wine was sour that contained such a high percentage of volatile acid. This soon became apparent to the taster himself and a new crop of difficulties arose. He wanted to be checked in his work and expected a determination of volatile acid (or as we commonly call it, "volatile") could be done almost as fast as he could taste wine and if he had 200 or 300 samples, he would expect them to be done in one day. He wanted them running day and night, Sundays and holidays, not,

of course because they were of any value but simply to prove his opinion and the poor, lonely wine chemist, harassed as he was, and upon trial, had very little satisfaction in finding one test, besides that of alcohol, which was of some value to his employer. But now other difficulties arose for the wine merchant. Pure Food Laws were coming into effect in European countries. Salicylic Acid (the common preservative used) had to be abandoned and the chemist was instructed to look for a preservative that could not be detected (and he is still looking). Then benzoic acid was adopted as a preservative, as benzoates, at that time, were very difficult of detection. Chemical science soon, however, caught up to them so that they were of no value. However, during this period, the chemist had become acquainted with the wine business and learned a few relations between composition of the wine to its keeping qualities and, therefore, was able to interpret an analysis. He could anticipate, to a certain extent, whether or not a wine would blow up, turn sour or spoil in some way when it reached a warm climate and, instead of finding a new preservative, concentrated his thoughts on getting the wine into such a condition that there would be no need of a preservative, and that a preservative would simply increase the cost of the wine and do it little, if any, good. This I think holds good in all food industries; that the need for preservatives decreases as our knowledge of the product increases. Through the efforts of the writer, wine was shipped without preservatives as an experiment. The first car was shipped out with many misgivings. It was expected that every barrel would blow up, and the entire car be lost. This was a very trying time for the chemist, as his theories were on trial and a failure meant a return to the old régime. The car, however, gave perfect satisfaction and nothing was heard afterwards. From that time on, the amount of preservatives decreased and methods for improving the vintage increased; in fact, a chemist's knowledge took the place of preservatives in this business. This was sometime before our national Pure Food Law. The firms, not employing a chemist, were found to be at quite a disadvantage, as their goods with preservatives, were subject to seizure, both by municipal and state authorities. This gave them undue notoriety and they

were forced to hire a chemist to improve and control their methods of manufacture and preparation of wine for the market. So much for the troubles and difficulties of pioneer chemists in the wine industry.

The chemist, having proved his value, now reached out to other things. He now started to look into the many causes of trouble. First, why some wines would not clarify, why wines of some localities and some districts degenerated, why some wines, shipped from the cellar in excellent condition, spoilt. These were the problems that confronted and still confront the wine chemist. He found out, however, that wines would not clarify, usually either on account of disease or on account of the composition of the wine, usually insufficient tannin. Then he started to improve the clarifying medium and to look into the reason of the degeneracy of wine after shipment. He found certain diseases which had to be, and were, overcome (these diseases, of course, never took place when preservatives were used, as the antiseptic action was too great for the micro-organisms to overcome). He then started to look into reasons of locality to find out why some cellars and some vintages turned out very poor wine. This, of course, was not original in California alone, as it was found in every other place throughout the world, and a great deal of literature on the subject was being printed so, while this did not take a great amount of original research, he acted as a medium to distribute scientific knowledge to the cellar superintendents, so that their methods of handling the vintage would be improved. This, also, at times, meant an outlay of money and is always, in corporations, a difficult thing to obtain. It was proven that wines, fermented at a high temperature, spoilt while those, fermented at a low temperature, nearly always kept sound. His labors then took on methods of controlling the temperature of the vintage, improving and handling, and also to ferment on cultivated yeasts of known virility and not leave the fermentation to chance. The quality of the wine, as well as its keeping properties were improved and the amount of spoilt wine reduced to a minimum. It is apparent that a successful business must turn out a uniform product. There is no difficulty in turning out a uniform sugar for example, but with wine, every

vintage is slightly different and, if a brand of wine is established, it is necessary to supply your customer with wine of exactly the same type. Otherwise, the consumer, being used to one wine objects to any other. The wine chemist has to help in the production of uniform products. As the blending of wines is the final operation (and as these blends compose at times 100 different wines) this is perhaps one of the most important duties of a wine chemist. Blends are usually made up in sample, analyzed, blended as near as possible to the composition of the previous blend of this type, and the blends are then distributed to the winery, which is to make them up and, after blending, are sent back to the laboratory where the chemist analyzes them again to check up and see whether these blends have been properly and uniformly made.

The following samples show the method of checking the blending. The analyses of the sample blend (made in the laboratory) and the actual blend (made in the cellar) must agree, otherwise, the blend is not uniform and must be reblended:

	Per cent Alcohol by Volume	Grams per 100 Cubic Centimeters			
		Total Acidity	Volatile Acidity	Reducing Sugar	Tannin
Winehaven Claret Blend No. 485 (265,000 gallons).					
Sample Blend ...	12.29	.500	.060	.145	.135
Finished Blend ..	12.37	.510	.060	.150	.140
Wahtoke Port Blend No. 482 (144,500 gallons).					
Sample Blend ...	20.78	.390	.043	6.60	.070
Finished Blend ..	20.78	.390	.046	6.63	.079

Besides this the wine chemist has duties in common with all chemists. He must analyze the water and soils of all the vineyards owned by his company, analyze the supplies, such as used

either in wines or in the vineyards, advise as to fertilizers to be used and devise means to gather as many by-products as possible.

I may say in conclusion that the wine chemist, in spite of temporary discouragements, is having more intimate relations with the Wine Industry. He is, in fact, becoming quite friendly, and he has hopes of being on the same good terms as his brothers in the sugar, dyeing, oil, petroleum, gas, soap and other industries, which the chemist has made famous.

INTERPRETATION OF THE RESULTS OF WINE ANALYSIS

By Charles S. Ash

San Francisco, California

Introduction.

It is evident that the analysis of any product is useless unless it can be correctly interpreted; in other words, every analysis that is made must be interpreted to be of any value. What the diagnosis is to the physician, interpretation of analytical data is to the chemist. Some of these interpretations are purely mathematical. The value of sugar cane is dependent on sugar content; ores are valued by the amount of metal they contain, and, therefore, assays are interpreted with little or no difficulty. The interpretation of analytical data on some other products are, on the other hand, most difficult and it is only by a careful study of many analyses of these products of known origin that we are able to show the true meaning of their chemical composition. In food products, we are quite content, as a rule, to tell from analytical data whether the food in question is pure or adulterated. As this interests most chemists, we will confine ourselves almost entirely to this question.

The interpretation of wine analysis has been confined almost entirely to the judgment of its purity; in fact, the only result of these interpretations has been the formation of a set of standards to which a wine must conform in composition to be considered pure wine. These hard and fixed standards do not accomplish the object of detecting adulteration on one hand while, on the other, they often work real hardship on wines of pure origin. Grapes of the same variety, grown in different soils and in different climates, produce wines of absolutely different taste, and composition. If this is true of the same variety of grapes, under different conditions, what must be the difference in composition of hundreds of varieties of grapes grown in almost every condition of soil and climate in the world! This we will discuss later.

There has been a tendency in the past, in writing of interpretations of wine analysis, to quote the interpretations of previous authors and this has influenced our already meager literature on this subject to such an extent that we have had but little original thought for many years, the last interpretation being only a compilation of previous data. To avoid such a tendency, the present writer will avoid previous literature on the subject, preferring to treat it from an independent viewpoint. I think you will pardon the writer for his temerity in taking this stand when he explains that yearly, for the past fourteen years, there has been received in his laboratory from 15,000 to 30,000 samples of wine of *known origin*. All these wines are examined and, at least, one-half analyzed.

We will, therefore, consider that the interpretation of wine analysis has two objects,—one to judge the purity of wine and the other to judge its quality and condition. We will take, under these two heads, part 1, dealing with adulteration and part 2, with condition, soundness and disease.

Part 1.—The object of all adulteration is to cheapen the article in question or to increase its commercial value by artificial and false means. No one will adulterate unless it is profitable and no one will substitute an artificial product, which costs more than the natural one. This, of course, is plain. In wine then, as in every other product, adulteration aims to decrease the cost of production or to increase its selling value. We, therefore, will consider the various forms of adulteration which may be used.

1.—Increase of volume.....	Addition of water Artificial wines Wines of foreign fruits
2.—Increase of strength.....	Addition of spirits and sugar, or both
3.—Increase of stability.....	Preservatives
4.—Improvement of appearance ...	Artificial coloring
5.—Changing of taste.....	Artificial flavor, saccharine, etc.

6.—Modified or fixed spoilt wine.

7.—To these forms of premeditated adulteration, we may still have adulteration which will come under the head of

accidental adulteration. Under this head, will come the presence of heavy metals, as zinc, iron, copper or arsenic.

Dilution.

Of all forms of adulteration, the most common, the most profitable and the most difficult of detection is dilution: the simple addition of water. The first two facts are self-evident. Why dilution is so difficult of detection needs some study. Having already touched on the effect of climate, soil and variety on the composition of the grape, we will go into further details. Now, it hardly seems necessary to state that grapes, grown in warm climates or grapes ripened to perfection, have more sugar than unripened grapes. It is also obvious that grapes that are unripe have a greater acid content than ripe grapes.

The following example shows the effect of climate on the composition of the juice of the same variety of grape:

Variety Carignan

	(1) Grown in France (Midi)	(2) Grown in Fresno, Cal.
Density at 60F.....	1.076	.0997
Total Solids, Grams per 100 c.c.	18.81	24.2
Reducing Sugar " " " "	16.2	23.26
Total Acids as Tartaric " "	.840	.590
Potassium Bi-Tartrate " "	.580	.376

The next example illustrates the effect of ripening on the composition of the grape juice:

(3)
Variety Zinfandel

	Sugar (% Balling)	Acidity as Tartaric
July 10	6	1.12
" 20	9	1.10
Aug. 1	14	.980
" 15	20	.700
" 20	23	.620

Now, the essential point I wish to bring out is this: that grapes, high in sugar content must necessarily be low in acid content and vice versa. Both conditions, that is, high sugar content and high acid content rarely exist in the same grape. On the other hand, low sugar content and low acidity also do not exist in the same grape. This is a generality. *This is very important.* It will, therefore, be seen that the resulting wine from grapes of high sugar content, will be high in alcohol and low in acidity, while wines, made from grapes low in sugar content, would give a wine low in alcohol and high in acidity. To repeat again, both high alcohol in a wine and high acidity, and low alcohol and low acidity, do not exist. We have, therefore, in cold countries the difficulty of obtaining grapes that ripen to a sufficient sweetness so as to give wine of high enough alcohol to preserve itself and a low enough acidity to be drinkable while, on the other hand, in warm regions, we have the reverse trouble of getting wines of high enough acidity and low enough in alcohol to have sufficient character and flavor to be considered desirable wine. These wines, as you see, are pure wines of absolute different composition and a hard and fixed standard made in either country to suit the conditions of the native wine may work hardship on the wines of the other country or, on the other hand, if the standard for example, happens to be based on the composition of the wine of the cold country, there would be little difficulty in diluting the wine from the warm country and still conform to the standard of pure wine.

I have spoken on the difficulty of a fixed standard. Let us take, for example, the American standard. This standard was meant to be, and is, a liberal standard, which tries to embrace all pure wines of the world. This standard is as follows:

	Red	White
Alcohol % by volume	7 to 16	7 to 16
Volatile Acid as Acetic, Grams per 100 cc	.14	.12
Reducing Sugar " " " "	.1%	1%
Ash	.16	.16
Sugar Free Solids " " " "	Not less than 1.60	1.40
Sodium Chloride " " " "	" more " .10	.10
Potassium Sulphate " " " "	" " " .20	.20

Let us say, for example, we have below an example of average, normal California Red Wine with the following composition:

Alcohol % by Volume	12%
Volatile Acid Grams per 100 cc	.100
Sugar Free Solids	2.50
Ash	.270
Sodium Chloride	.005
Potassium Sulphate	.015

This wine can safely be diluted one-half (2–3rds wine and 1–3rd water) and still be able to conform to these standards. The composition of the diluted wine would then be as follows:

Alcohol % by Volume	8%
Volatile Acid, Grams per 100 cc	.067
Sugar Free Solids	1.67
Ash	.180

This comes well within the standard of purity as laid down by our government. On the other hand, these standards will work hardship on some of the finest old wines produced. This we will discuss when we take up the question of Volatile Acid of the wine, as it is out of place under this heading.

To return; we have shown what can be done under the standards laid down by our government. There is nothing in these standards showing the relation of alcohol to total acidity, much less showing the composition of this acidity in natural wine.

In this case cited, the original acidity of the undiluted wine was .6 grams per hundred cubic centimeters. When this wine is diluted, we find an acidity of .4 grams per hundred cubic centimeters. *A natural wine with an acidity of .4 grams per 100 c.c. and an alcoholic strength of 8% by volume does not exist.*

The obvious question is then asked: why a diluted wine cannot be acidified to raise the acidity to that of normal wine of such an alcoholic strength. This certainly complicates matters. The only available acids are, as we know, Citric and Tartaric Acids. As Citric Acid is never present in grapes in quantitative amounts, its presence in most cases will be indicative of manipu-

lation. We have now only Tartaric Acid. The natural non-volatile acidity (fixed acidity) of wine is largely made up of Potassium Bi-tartrate and not Tartaric Acid. Free Tartaric Acid is only present in small quantities in natural wine. So, if Tartaric Acid was added to this diluted wine we will have a larger amount of free Tartaric Acid than Potassium Bi-Tartrate. We cannot use Bi-Tartrate to acidify the diluted wine, as it is only sparingly soluble. The following table is explanatory:

	Natural Wine	*Diluted Wine*	*Diluted Wine Acidified*
Alcohol % by volume	12.0	8.0	8.0
Total Acidity (as Tartaric) Grams per 100 c.c.	.600	.400	.800
Volatile " (as Acetic) " " " "	.100	.067	.067
Sugar Free Solids " " " "	2.50	1.67	1.67
Ash " " " "	.270	.180	.180
Potassium Bi-Tartrate " " " "	.300	.200	.2000
Free Tartaric Acid " " " "	Trace	Trace	.400

Here, in the natural wine, we have .3 grams Potassium Bi-Tartrate and a trace of Free Tartaric Acid, and in the diluted wine, we have .2 grams Bi-Tartrate, while in the diluted acidified wine, we have .2 grams Bi-Tartrate and .4 grams Free Tartaric Acid; the Free Tartaric Acid, being in excess of the Bi-Tartrate, while it should only be one-third at the most. Now, suppose we add the alcohol % by volume and the total acidity (grams per litre). We have in the natural wine a total of 18, in the diluted wine 12, and in the acidified wine 16. Whenever a Red Wine has a total acidity of less than 16, it should be investigated. All this data is relative to the effect of dilution on acidity. I know the difficulty of showing everything in one example, but we have already gone to greater length than I had wished to go. It is obvious to us all that dilution will lower the percentage of solids, the percentage of ash and other constituents of the wine. I might state that the alkalinity of ash, figured in terms of Bi-Tartrate should approximately equal the percentage of Bi-Tartrate found in the

original wine; in other words, the alkalinity is almost due entirely to the Bi-Tartrate which is converted into Potassium Bi-Carbonate by incineration. In conclusion, it would appear that California red wines, having a sugar free solids less than 2.30 in conjunction with an alcohol plus acid total of less than 16, is to be regarded with suspicion. With white wines, a sugar free solids of less than 1.60 and alcohol plus acid total of less than 15, should also be looked upon with suspicion. Wines, having a large amount of Free Tartaric Acid, in proportion to the Potassium Bi-Tartrate, and coupled with low sugar free solids would indicate that the wine had been diluted and acidified. The relation of Potassium Bi-Tartrate to the free Tartaric Acid is the same in white wines as it is in reds.

Artificial Wines

These wines are made by fermenting sugar, sucrose or glucose, either alone or thrown over grape pomace. They are, in consequence, very light in color or colorless, and have either to be blended with natural wines or colored artificially. They are also high in free Tartaric Acid and low in Bi-Tartrate and low in solids and ash. They often have no Potassium Bi-Tartrate at all, the acidity being due to free Citric or Tartaric Acid. Those made from glucose are always high in solids, due to dextrin or other unfermentable substances. Such substances are too easy of detection to be mentioned here. Those made from sucrose are always a superior article. They are, however, very expensive and in countries where grapes are cheap (as in California) cost more than the natural wine. For example, sucrose cost, say, 5c per lb.; grapes, having a sugar percentage of 22 per cent will have 440 lbs. of sugar per ton of grapes; 440 lbs. of sugar, in turn, at 5c. per lb. would cost $22 and, therefore, sugar would be equal to grapes costing $22 per ton. The average price of grapes in California, year in and year out, will be from $12 to $30, the average cost being somewhere around $18. Now, it is obvious that no one would use this form of adulteration unless the price of grapes were well over $30 per ton. In colder countries where the grapes do not ripen to a sufficient sugar

percentage, the sucrose is very often added to give the resulting wine sufficient alcohol to preserve it. When this is not done to excess, it is almost impossible (without a thorough knowledge of the composition of natural wines of such district) to show this form of adulteration. Sucrose is inverted by the action of the yeast and acid, before fermentation. The inverted sugar is very closely allied to that of the natural sugar of the grape. I might say in conclusion, all the artificial wines, that the writer has seen, have come, of course from localities where grapes were dear. Their acidity has been due almost entirely to free Tartaric or Citric Acid. They are often artificially colored and preserved with some preservative. Most of these have been made from glucose. Sometimes sweet wines, like Port, have been made. These wines contain about 12 per cent alcohol and as high as 20 per cent of total solids, the method of making being that the glucose solution has fermented until fermentation takes place no longer and the wines stick. Preservatives are then added and the wine is colored; sometimes synthetical flavors are also added. These wines never cloud and give no trouble, during changes of temperature in climates, that all natural wines do.

Foreign Fruit

This form of adulteration I hardly think exists in the state of California on account of the low price of grapes and the comparatively high price of other fruits. In Europe, apples and figs have been, at times, fermented and mixed with natural wines. Such wines, by themselves, would easily be detected, as very few other fruits, beside the grape, contain any Bi-Tartrate. The absence of Bi-Tartrate or a very low percentage of same in a comparatively normal composition otherwise, would tend to show this form of adulteration. When more light is shown on the acid composition of various fruits, no trouble will be experienced in detecting such adulteration.

Increase of Strength

This is done by the addition of sugar, as had already been described, or spirits, or possibly both. The addition of spirits

cannot take place in American dry wines, as wines must have 4 per cent of total solids to be eligible to fortification. Neither can taxpaid spirits be added to such wines, for it is against the revenue laws to mix taxpaid and free spirits. Such a form of adulteration would be criminal and liable to prosecution. Some countries allow spirits to be added to wines for exportation. This is readily detected on account of its abnormally high alcohol together with high acidities. The addition of spirits and then dilution by water afterwards, would make a double dilution and would be detected under means already described in the paragraph on dilution.

Increase of Stability

Whenever wines have been diluted or made from unsound material, preservatives are sometimes added to give them keeping qualities, without which they would get progressively worse, so as to be impossible to market them. In this connection the use of preservatives, irrespective of whether they are harmful to the human system or not, should be prohibited in the wines for the reason that poor or putrid articles could be marketed, which would be impossible to do without their use.

Improvement of Appearance

Whenever artificial or imitation wines are sold, either alone or mixed with natural wines, the color is insufficient to meet the popular demand. It is, therefore, imperative that these wines be given the appearance of normal wines. To do this, they must be colored. Aniline colors are used for this purpose almost entirely, as vegetable colorings are not fast and either fade or deposit when subjected to daylight. The usual clarifying methods (the addition of albumen) will very often deposit vegetable colors and, therefore, after wine has received a clarification, vegetable colorings are almost entirely removed from the wine. The above statement, in reference to the coloring of wines, is applicable to red wines entirely. White wines are rarely colored, though in isolated cases, the writer has seen wines given green tints by artificial means.

Change of Taste

Sometimes flavors are added to natural wines to improve the state, or give them either the appearance or taste of old wines but, in most cases, this is a waste of money. Such form of adulteration is very difficult of detection. However, a higher percentage of esters than is normal in a wine, especially volatile esters, will tend to show adulteration of this form. Saccharine has been at times added to white wines to imitate wines of the Sauterne type. This is very simple of detection, 1st—by the method of detecting saccharine itself, and, 2nd., that the total solids of such a sweetened wine is no higher than the normal solids of a dry white wine. Such sweetened wines are also very low in reducing sugar and, therefore, cannot derive their sweetness from natural sources.

Modified or Fixed Spoilt Wines

This is the last form of premeditated adulteration. Spoilt wines or sour wines, which have either been made from spoilt grapes or have been spoilt by neglect or degenerated by some disease, are often marketed after manipulation. The common method of doing this is to neutralize the excess of acidity, either by potassium, calcium or magnesium salts—usually calcium carbonate is used to do this work. The resulting ash of such wines is very high in lime salts, the total ash going considerably over 1-10th of the sugar free extract. The fixed acidity is usually very low in comparison to the volatile acidity. This is due to the fact that such manipulation is rarely, if ever, successful, and wines, having been neutralized in this way, usually are only temporarily checked; the disease continues and more volatile-acid is generated; the neutralization only neutralizing the acids present at the time of neutralization.

We now come to the final form of adulteration, which we will call Accidental Adulteration. That is the presence of small amounts of heavy metals in wine. The presence of copper, tin or zinc is due to the pipe line, which the wine is run through. The amounts are very low indeed and it is almost impossible to avoid these contaminations. Carelessness, however,

in allowing wines to stand in pipes until they corrode will very often increase this small percentage to an enormous amount. The presence of arsenic in wine can rarely be considered accidental adulteration, as it is a natural constituent of grapes in some localities. The largest amount ever seen by the writer in California wines is one part in four million: usually one part in twenty to fifty million is normal. Arsenic occasionally is added to the wines, by the use of sulphur in fumigating casks. This, of course, would be considered accidental adulteration.

I wish to conclude, as I have begun, by wishing to avoid a fixed standard applicable to all wines in the world. There is only one way which we can tell with approximate certainty whether a wine is pure or adulterated and this is to have for our standard of pure wine the same standard as the country from which the wine originates; for example, standards set down by the Swiss government for Swiss wines, should be applicable to Swiss wines; those set down by the German government for their Rhine and Moselle wines, and other districts, should be applicable to wines of that district; for French wines, those set down by the French government as standards for their natural wines of different districts, should be used on such wines. For American wines, standards should be made by the United States government or by the Pure Food Authorities of the different states where the wine is grown. In this way, we will not demand the same composition for Algerian wines as we do for German wines, nor the same for Swiss wines as we do for California wines. This I believe will be a very simple matter and, in my opinion, is the only way of controlling this question. A unification of analytical methods would also be a big factor, and should be urged by such bodies as this Congress. If not, we must follow the analytical methods of the country in question, that is, if we are examining French wines, we must realize that these standards have been based upon methods of official French analysis, and results, using any other methods, will either work hardship or may defeat the enforcement of a Pure Food Law.

UNDER THE HEAD OF SOUNDNESS Part 2.

Just one word, before I close this already too lengthy paper, in regard to unsound and diseased wines. Such wines can be made from grapes and be pure in every way and still be unfit for human consumption. A parallel case would be in any putrid, decayed or deteriorated food product.

This may be due to the following reasons, as the manufacture of wine from rotten or diseased grapes, or the wines may be made from sound grapes, and become diseased from improper methods of fermentation or neglect. To be brief, we will suppose that the Must contains

Sugar	20 grams per 100 c.c.
Acidity as Tartaric	8–10 grams per 100 c.c.

The corresponding wine, fermented under normal conditions, would have a composition somewhat as follows:

Alcohol	11.5 per cent by volume
Total Acid	.600 per cent
Volatile Acid	.060 per cent
Reducing Sugar	.120 per cent

However, if this wine should be fermented badly, or the temperature rise too high, so that the yeast is either killed or lies dormant, secondary fermentation will set in, and we will have a wine of quite different composition, depending on the severity of conditions or neglect in handling. A typical composition of such a wine would be as follows:

Alcohol	11 per cent by Volume
Total Acid	.700 per cent
Volatile Acid	.200 per cent
Reducing Sugar	.500 per cent.

Notice the difference of these two results made from the same Must. Let us go backward and say that we have a spoilt wine with this composition and see what we can tell about it from analytical data. In the first place, notice that this wine has a high volatile acid. This shows that it has been attacked by

micro-organisms and that secondary fermentation, in consequence, has set in. The product of such fermentation (the high volatile acid) is present. We then look at the reducing sugar and find that it is high. We see that the conversion of sugar into alcohol has not been completed and this confirms our opinion of secondary fermentation. The wine is absolutely spoilt and should not be used under any conditions. This wine should not be blended with any other wine, as it would simply contaminate the entire blend. A great many mistakes are being made in trying to work off wine of this kind in small quantities. This is an absolutely bad practice, for nine times out of ten, the entire blend is ruined. It is always best to make your first loss at once. Wine of this type, of course, would make excellent wine vinegar, and could be used for such purpose. In well-controlled wineries, wine of this type is reduced to a minimum

We will now follow new, sound wine over a period of years and see what changes we may expect in the volatile acid content.

	Dry Red or White	Sherry	Port, Angelica Muscat, etc.
Sound new wine	.05 to .08	.04 to .08	.03 to .05
Sound wine 1 year old	.06 " .100	.06 " .08	.04 " .06
Sound wine 3 years old	.09 " .120	.08 " .100	.05 " .07
" " 5 " "	.100 " .140	.100 " .120	.07 " .09
" " 8 " "	.120 " .160	.120 " .160	.08 " .100
" " 10 " or over	.140 " .170	.140 " .180	.100 " .120

This represents the natural increase of acidity one would expect. This is not intended as a fixed standard, but rather a guide. From this it is seen that a young wine, which would be condemned as spoilt, may be a perfectly fine sound old wine.

EXPERIMENTS ON FEEDING GUINEA PIGS "SALTS OF TIN" IN MEASURED QUANTITIES FOR SEVERAL WEEKS

By H. A. Baker
New York

Seven normal young Guinea Pigs, of average weight of about 257 grams, were put in separate pens and was each fed daily, except Sundays, one Gelatin Capsule containing 12.6 milligrams of tin in the form of Hydrates mixed with Corn Meal.

The capsules were prepared in the following way:

Four grams of pure tin were dissolved in a small amount of Hydrochloric Acid and evaporated almost to dryness. This was then neutralized with Sodium Carbonate and dried, after which it was mixed with fine Corn Meal. 55,000 milligrams of the mixture contained 4000 milligrams of tin.

201 Gelatin Capsules, weighing 11.438 grams, were filled with this Corn Meal mixture and found to weigh 46.281 grams, so that each full capsule weighed 230.25 milligrams. Subtracting the weight of the empty capsule, 56.9 milligrams, we have as the contents of each capsule 173.35 milligrams of Corn Meal mixture, which contained 12.607 milligrams of tin.

These capsules were all standard size so that we consider the amount of tin in each capsule to be the same, especially since the amount of Tin Salts in the mixture was small.

The capsule was administered to the Guinea Pig by forcing it down his throat, in order to make sure that the proper dose had been given.

The Guinea Pigs were given an ordinary diet of Carrots, Lettuce and Crackers.

As will be observed from the table appended, the Guinea Pigs showed different resistance toward this chemical. Five of the Guinea Pigs were fed until death occurred. They lived the following number of days: 9, 10, 19, 19, 22, respectively. Two

other Guinea Pigs which had taken a capsule a day for twenty-five days lived, but were after that fed no more capsules. Twenty-three days after these two Guinea Pigs had been fed no more capsules, they suddenly died from exposure from an open window.

In addition to observing the doses which were fatal to these Guinea Pigs, their livers were analysed in order to see whether or not any tin had become stored up there. Examination showed the livers of the dead Guinea Pigs to be somewhat bleached on the outer edges.

It can be observed from the table that these Guinea Pigs accumulated in their livers a small amount of tin each day, as long as the dose was administered. This rate of accumulation was about $\frac{1}{10}$ of a milligram of tin per day; the average rate for five Guinea Pigs being .113 milligrams of tin per day.

Using this rate as a basis for calculating the amount of tin in the livers of the Guinea Pigs numbers six and seven, at the end of the twenty-fifth day, we would have 2.827 milligrams of tin present in each liver. At the end of the twenty-third day, during which period no more tin had been fed, we still found .3 and .5 milligrams of tin in the livers of Guinea Pigs numbered six and seven respectively, which means that the tin had been eliminated from their livers at the average rate of .1099 milligrams and .1011 milligrams per day respectively. From the experiment, of course, it cannot be known that this average rate of elimination was the actual rate, and it probably was not. It is probable that the rate of elimination varied according to the concentration of the amount of tin in the livers of the Guinea Pigs and fell off as the concentration decreased.

It may be observed from the table that the average absorption in the livers of the amount of tin fed to the Guinea Pigs was .898%.

These individual doses were very large, and no other experiments have been carried on to determine what the effect of smaller doses for longer periods of time would have been.

The exceedingly small amounts of tin involved in the determinations were estimated by a very delicate iodimetric titration method, using N–100 Iodine. We consider the accuracy of

this titration to be within 3-10 of a cc, which means that the determinations are within .2 milligrams of accuracy, probably always tending somewhat toward high results.

TABLE SHOWING RESULTS AND ANALYSES FROM EXPERIMENTS ON FEEDING GUINEA PIGS "SALTS OF TIN" IN MEASURED QUANTITIES

By H. A. Baker

	GUINEA PIGS						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
No. days fed Salts of Tin	9	10	19	19	22	25	25
Amount in Milligrams of tin as "Salts of Tin" fed each day	12.6	12.6	12.6	12.6	12.6	12.6	12.6
Total Tin fed as "Salts of Tin" in Milligrams	113.4	126.0	239.4	239.4	277.2	315.0	315.0
RESULT	Death	Death	Death	Death	Death	Living	Living
Weight of Liver in Grams	12.7	9.8	10.0	8.9	10.0	9.5	9.8
						(Determined 23 days later)	
Weight of Pig in Grams	260	215	251	298	306	248.5	220
Weight of Liver as percentage weight of Pig	4.88	4.56	3.98	2.99	3.27	3.82	9.46
Tin found in Liver in Milligrams	1.05	1.015	2.30	1.80	2.90		
Rate of accumulation of tin in liver per day in Milligrams	.1166	.1015	.1210	.0947	.1318		
Percentage total amount of tin found in liver	.926	.805	.961	.752	1.045		

GUINEA PIGS NUMBERS SIX AND SEVEN

These were fed no more "Salts of Tin" after 25 days, but at the end of 23 days more they died one night from exposure to cold from an open window.

	Guinea Pig No. 6	Guinea Pig No. 7
Estimated milligrams of tin in liver at end of 25 days, calculated from average rate of accumulation of tin in livers of other five Guinea Pigs..	2.83	2.83
Milligrams of tin found in livers after 23 days rest from feeding "Salts of Tin".....	.30	.50
Average rate of elimination of tin per day in milligrams.....	.1099	.1015

No physiological examination was attempted, so that the conclusions from this work are somewhat limited. The following deductions, however, would appear to be patent:

First.—That daily doses of 12.6 milligrams of tin as Hydrates were fatal to the Guinea Pigs in from nine to twenty-five days upwards.

Second.—That tin was progressively accumulated in the livers of these Guinea Pigs when fed daily doses of 12.6 milligrams of tin as Hydrates.

Third.—That the tin accumulated in the livers of the Guinea Pigs by feeding, as above noted, was eliminated rather slowly after feeding was discontinued.

It is desired to give credit to W. S. Sellars who performed the major part of the Laboratory work in connection with this experiment.

SPECIAL ADAPTATION OF IODINE TITRATION METHODS FOR THE ESTIMATION OF TIN, ESPECIALLY IN CONNECTION WITH DETERMINATIONS OF "SALTS OF TIN" IN CANNED FOODS

By H. A. Baker
New York City, N. Y.

This method being an adaptation of parts of several standard and well known methods, no particular reference will be made to the sources of the principles employed in the method.

The tin in the canned food products is obtained as a sulphide precipitate from wet combustion, with Nitric and Sulphuric Acids, of 100 grams food product.

The clear Sulphuric Acid residue is diluted, neutralized with ammonia, and then rendered about 2% acid with Hydrochloric Acid, after which it is thoroughly saturated with Hydrogen Sulphide Gas. This precipitate is then filtered on a Gooch Crucible with a false bottom. The precipitate may contain foreign substances, such as Lime, Phosphorus and Silica, some Lead, or even small amounts of Iron, but none of these will cause any trouble subsequently in the titration so that the labor of separating the tin completely from the precipitate is obviated.

After washing the precipitate three or four times in a Gooch Crucible, it is transferred to a small porcelain dish by simply forcing out the false bottom of the Gooch Crucible and its asbestos pad and rinsing off the crucible.

The precipitate, mixed with asbestos, is now transferred to a 300 cc. Erlenmeyer Flask and boiled with strong Hydrochloric Acid; Potassium Chlorate being added from time to time to insure the complete breaking up and solution of the tin sulphide, as well as the elimination of the sulphur. This is accomplished in a very few minutes. A few strips of pure aluminum foil, free

from tin, are then added to the flask until all of the Chlorine is eliminated. This flask is then attached to a large Kipp Apparatus, charged with pure marble and Hydrochloric Acid, delivering Carbon Dioxide. The Carbon Dioxide from the Kipp Apparatus is passed through a Scrubber, then led into the Erlenmeyer Flask through a bulbed tube in the rubber stopper of the Flask, delivering the Carbon Dioxide near the surface of the liquid in the flask. It is led out of the flask through a second bulbed tube, the opening of which is near the top of the flask, and the Carbon Dioxide gas escapes from the end of a glass tube about 10″ long, immersed in water about 8″ deep. This gives a water seal to the delivery tube and a pressure against which the Kipp Apparatus must work. This obviates any violent flow of the gas when not desired and permits a gas pressure in the Erlenmeyer Flask.

Pure seamless black rubber tubing and $\frac{3}{8}$″ glass are used to form the connections specified.

When the flask is thus attached to the Carbon Dioxide Insulating Apparatus, as above mentioned, the air from the flask and the tubing connections is first thoroughly dispelled by lifting the delivery tubes out of the water cylinder seal so that the Kipp Apparatus has practically no pressure to overcome. A large amount of Carbon Dioxide is thus forced through the system and air is completely distilled. The rubber stopper in the Erlenmeyer Flask is then raised, and about one gram of Aluminum Foil is dropped into the flask. This quickly reduces the tin to the metallic form and evolves a great deal of Hydrogen Gas.

The flask is then placed on a hot plate and heated to boiling. The aluminum disappears and the tin is changed into Stannous Chloride. After a few minutes boiling, the flask is set off the hot plate and then cooled in ice water, while still under Carbon Dioxide insulation.

When the flask is first attached to the Kipp Apparatus, enough water is added to dilute the Hydrochloric Acid so that its strength is approximately 30 to 40%. After the addition of aluminum foil and boiling, the acid strength will be approximately 25 to 30%.

After cooling, as above mentioned, the contents of the flask are ready for Iodine titration. This may be accomplished by two methods; an excess of Iodine may be run into the Erlenmeyer Flask directly by simply lifting out the rubber stopper and running in the Iodine solution while Carbon Dioxide is issuing from the flask. The excess of Iodine must then be titrated back with Sodium Thiosulphate. Usually, however, it is satisfactory to simply detach the flask from its rubber tubes, wash down the tubing, rubber stopper and sides of the flask with some air free water, add starch paste and titrate directly and quickly with N–100 Iodine solution until a faint blue color is obtained. The asbestos which is in the flask will not interfere with this titration.

The air free water is made by boiling distilled water, adding a small amount of Sodium Bicarbonate and then a slight excess of Hydrochloric Acid.

Only one sample has been mentioned so far, but duplicates are always run together, as the Kipp Apparatus is arranged to handle two flasks at a time, simply by dividing the Carbon Dioxide Gas by means of a "U" tube and connecting an arm to each Erlenmeyer Flask. It is not only desirable but necessary with this method, as with practically all titration methods, to run duplicate samples. The N–100 Iodine is standardized against pure tin solutions or food mixtures, such as Apple Butter, containing a known or added amount of salts of tin.

The advantages which may be found in this method are:

First:—Only one filtration is required and that can be performed very quickly on account of the fact that it is performed under suction.

Second:—No perfect separation of the tin from other metallic precipitates or impurities is necessary.

Third:—There is no delay at any point in the method, such as long washings, waiting for filter paper to dry, or the loss of time over very slow and careful burning of the precipitate.

Fourth:—The titration reading gives the amount of tin directly, as no corrections are involved.

Fifth:—In the hands of competent operators, many more

analyses can be performed in a given time than with a Gravimetric method.

Sixth:—The accuracy of the method is very satisfactory, being at least as accurate as any Gravimetric method.

Seventh:—This same method may be used directly for metals containing tin without previous separation. Metals, such as tin plate, solders, Babbitt Metals or Composition Metals may be dissolved up directly with Hydrochloric Acid in an Erlenmeyer Flask attached to the Kipp Apparatus and titrated precisely as above described, by either direct titration method or the excess method, using, of course, a strong Iodine solution. Aluminum, Zinc, Iron, Lead, Antimony, Bismuth or small quantities of Copper do not interfere with the method. When large quantities of metals are dissolved directly in the Erlenmeyer Flask, the addition of Aluminum foil is not necessary. If metals are dissolved up in contact with the air, or Potassium Chlorate is necessary for complete solution, they may be reduced with aluminum foil and then handled exactly as previously described.

"SPRINGERS" IN CANNED FOODS—CAUSES AND PREVENTION

By H. A. Baker
New York, N. Y.

"Springers" is a trade term given to cans with bulging ends which contain perfectly sound and sterile food products. They are undesirable because the easiest test for the Housewife to apply, to tell whether the container is sound, is to observe that the ends of the cans are flat or drawn in slightly. It is desirable that this test should always be applicable and sufficient. Therefore, canned foods should be so packed that no "Springers" will be formed.

When a can is a "Springer" there is too much gas in it or not enough space to hold the gas under negative pressure at all weather temperatures.

The gases in the head space of these "Springers" are never more than three; Carbon Dioxide, Nitrogen and Hydrogen. Very often no Hydrogen is found. Oxygen is practically never found.

The Carbon Dioxide is formed in practically all canned foods during the time of processing. It is also formed excessively if food products are not worked through quickly from the beginning of their preparation to their final sterilization. This is true particularly of fruit and vegetable products after they have been peeled or their cells have been broken in any way or have been subjected to heat.

If food products are allowed to stand in containers before sterilization, an excessive amount of Carbon Dioxide Gas is formed.

Nitrogen Gas is simply a residue from unremoved air. Hydrogen Gas, when formed, is the product of attack of fruit or vegetable acids on the metallic container.

Changes in temperature of these cans produce changes in gas

pressure. At 85° Fah. we may have a well puffed can, at 60° one in which there is practically no pressure and at 45° to 50° there will be a vacuum. These changes occur with a decrease of temperature because the gas itself contracts, the solid and liquid contents of the can contract, and the solubility of the gas is increased.

"Springers" are usually warm weather phenomena.

The general history of the formation of gas and its behavior in a can are as follows:

First:—A certain amount of air is left in the can at the time of sealing, even if the cans have been "exhausted."

Second:—A certain amount of Carbon Dioxide is formed during the processing or cooking of the food products. If they have been allowed to stand after being prepared, an excessive amount is formed. The Carbon Dioxide is usually 8 to 15% of the gas in the head space at ordinary temperatures. At higher temperatures, more of the gas comes out of solution and can be found in the head space.

Third:—The oxygen left in the can disappears, either by combination with some element of the food product, such as Butter Fat in Milk, etc., or by combinaton with metal, or by combination with Hydrogen Gas formed by the action of Organic Acids on metal.

All three of these reactions have been traced. This withdrawal of Oxygen in the can tends to give the can a temporary vacuum.

Fourth:—When organic acids are present, Hydrogen Gas is formed in plain tin cans and helps to make pressure.

Fifth:—The influence of the increase of heat on the expansion of the solid, liquid and gaseous contents of the can, and the decrease of solubility of the Carbon Dioxide Gas are usually responsible for the appearance of "Springers."

Sometimes Hydrogen Gas Springers are formed which are usually old samples and could not be classed as temperature springers.

If very small head space is left, it requires but a slight expansion of the contents to change the contour of the can from a flat to a bulging condition.

These difficulties can be obviated if the following points are observed:

First:—Sufficient space must be left in the can to receive the gases which will be formed. This means evenly filled cans in which the exact amount of head space has been determined for each food article and process.

Second:—This head space must be "exhausted" adequately so that enough vacuum is left to receive the gases that will be formed and still leave the ends of the containers drawn in or under vacuum.

Third:—Cans, after sealing, must be processed as soon as possible to minimize the formation of Carbon Dioxide Gas, and there should be no undue delay in working the food product through the factory from the beginning of its preparation until it is sterilized.

Fourth:—With highly acid food products, the metallic container should have a protective coating of enamel.

APPARATUS FOR QUANTITATIVE EXTRACTION OF THE GASES IN CANNED FOOD CONTAINERS

By H. A. Baker

New York City, N. Y.

Figure # 1 shows the apparatus ready for use.

Figure # 2 shows the apparatus actually in use, with the gas being collected in a regular gas burette.

The apparatus consists of an extensible strap iron frame in which a can may be set and clamped down by means of a screw clamp.

Entering at the base of this steel frame is a hollow steel needle which is observable in figure # 1. The rubber stopper shown alongside of this needle, in use, is placed over the needle. It is of such height that the rubber stopper must be considerably compressed before the steel needle punctures the bottom of the can. The steel needle is connected with a water supply in the cylinder having air pressure in its top. A stream of water, under about fifteen pounds pressure, can thus be forced through the puncturing needle into the can. Adequate water pressure from any other source would be satisfactory.

The strap iron holder, which is either screwed or clamped on to a table, has a twisted iron shank so that it tips at an angle of approximately 45°. This places the can in such a position that it can be punctured and the gas drawn off from its highest point as is shown in figure # 2.

In this figure, a regular Doremus Gas Extracting Apparatus may be seen.

Any other kind of puncturing arrangement, based on the same principle, would answer satisfactorily.

This clamp has a screw compression which works on the bottom of the can, and a hollow steel puncturing needle through which the gas is extracted from the top of the can. The puncturing needle is enclosed in a rubber stopper so that compression

and tight connection is necessary before the steel needle punctures the top of the can.

The top of the gas extractor is connected with a regular gas burette by means of capillary tubing with rubber connections.

The complete procedure for extracting the gas is as follows: 1st—After the can is in place, water is forced through the steel puncturing needle in the base, and while the water is still flowing through, the screw clamp from the top is turned down until the compression in the rubber stopper is great enough to allow the needle to puncture the base of the can. Water under pressure may then enter the can. The Doremus clamp is screwed down solidly, the hollow steel needle filled with water, the capillary connecting tubes between the gas burette and the extractor are filled with water and connected, as shown in figure # 2. The clamps on the rubber connections are then loosened, the gas burette opened and the Doremus clamp screwed down until the can is punctured. The gas then flows out through the capillary tubing, displacing the water in the gas burette.

When all of the gas has been removed, some of the liquid in the can will come over and sweep any gas in the capillary connections into the gas burette so that quick and complete extraction of the gas from the head space of the sample is obtained.

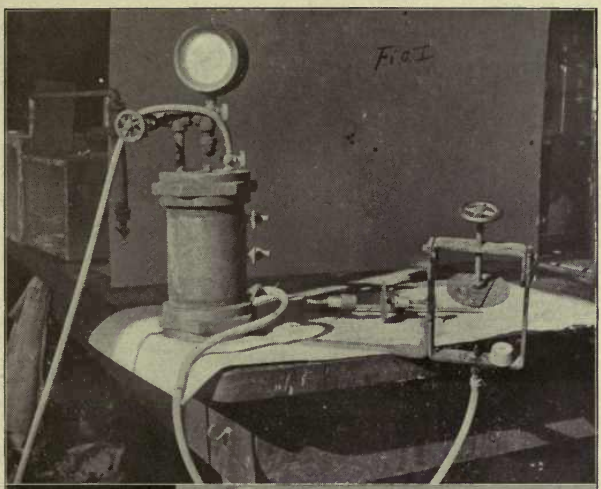


Fig I

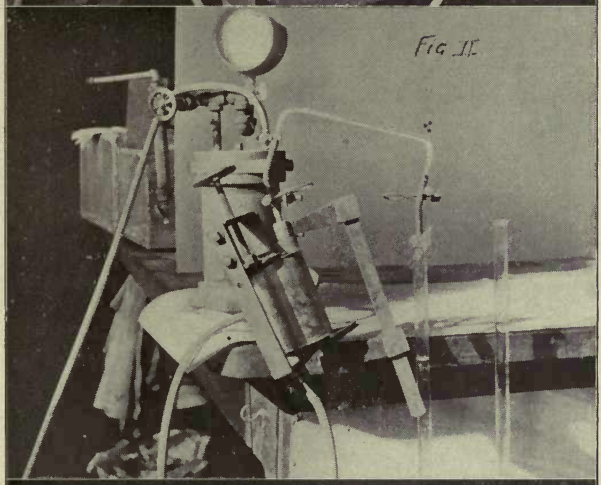
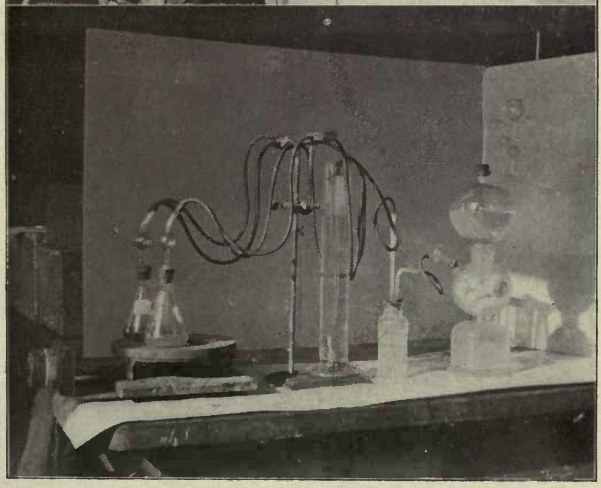


Fig II



THE DISAPPEARANCE OF OXYGEN IN CANNED FOOD CONTAINERS

H. A. Baker
American Can Company, New York, N. Y.

There is always left a head space in the top of every can in which food is packed. In this head space or chamber there is always more or less air, even when an "exhaust" has been used to withdraw the air. The analyses of probably 100 samples of gas from these head spaces from sound cans containing nearly all kinds of food products have practically never shown any oxygen content. Slight traces have been found in cans containing foods of less than two months age.

The analyses usually show Carbon Dioxide 8 to 15% with the balance Nitrogen Gas. Hydrogen is also found to be present in some instances, particularly with acid fruits.

It might be presumed, in many instances, that the Oxygen combined directly with the food product in such oily foods as Salmon, Evaporated Milk, Pork and Beans, etc., but the absence of Oxygen in all classes of canned foods calls for another explanation, and in order to trace the disappearance of Oxygen and the appearance of Hydrogen Gas, sample cans were prepared containing distilled water, dilute solutions of Citric Acid and Salt and their gas changes were followed by analyses.

The analyses of many samples of gas, drawn from cans containing food products, have shown the presence of Hydrogen Gas, particularly where acid fruits were concerned. This gas does not usually appear until after the foods have been packed two or three months, and inasmuch as some corrosion has necessarily been taking place during this time, the question arises why Hydrogen Gas does not appear more quickly.

The following experimental pack and analyses were made in an attempt to find out the facts.

Ten regular No. 2 cans were filled as follows:

Two cans $\frac{5}{6}$ full with 500 cc plain distilled water.

Two cans $\frac{5}{6}$ full with 500 cc solution containing $\frac{1}{4}\%$ Citric Acid.

Two cans $\frac{5}{6}$ full with 500 cc solution containing $\frac{1}{4}\%$ Citric Acid and $1\frac{1}{2}\%$ Sodium Chloride.

Two cans $\frac{1}{2}$ full with 300 cc solution containing $\frac{1}{4}\%$ Citric Acid.

Two cans $\frac{1}{2}$ full with 300 cc solution containing $\frac{1}{4}\%$ Citric Acid and $1\frac{1}{2}\%$ Sodium Chloride.

These cans were sealed cold, leaving head spaces of approximately 100 cc and 300 cc of air at room pressure and temperature in the cans filled with 500 cc and 300 cc liquid respectively. They were cooked one hour at a temperature of 245° Fah. and then allowed to cool in the air. This was done on October 9th, 1911.

Analyses of the gas from five of the cans were made 42 hours afterwards; one analysis 21 days afterwards and four analyses $8\frac{1}{2}$ months afterwards.

The following are the gas analyses obtained:

1. Can $\frac{5}{6}$ full containing 500 cc plain distilled water, sealed cold, and processed one hour at 245° Fah.—after standing 42 hours had a gas content of the following analysis:

Carbon Dioxide	Trace
Oxygen	13.20%
Hydrogen	.00%
Nitrogen	86.80%

2. Duplicate of this sample, after standing $8\frac{1}{2}$ months, gave the following analysis:

Carbon Dioxide	Trace
Oxygen	.00%
Hydrogen	.00%
Nitrogen	100.00%

3. Can $\frac{5}{6}$ full containing 500 cc of $\frac{1}{4}\%$ Citric Acid solution, sealed cold, processed one hour at 245° Fah.—after standing 42 hours had a gas content analyzing as follows:

Carbon Dioxide	.70%
Oxygen	9.65%
Hydrogen	.00%
Nitrogen	89.65%

4. Duplicate of this sample, after standing 21 days, gave the following gas analysis:

Carbon Dioxide	.85%
Oxygen	.40%
Hydrogen	.00%
Nitrogen	98.75%

5. Can $\frac{5}{6}$ full with 500 cc solution containing $\frac{1}{4}$% Citric Acid and $1\frac{1}{2}$% Sodium Chloride, sealed cold, processed one hour at 245° Fah.—after standing 42 hours had a gas content of the following analysis:

Carbon Dioxide	.65%
Oxygen	9.05%
Hydrogen	.00%
Nitrogen	90.30%

6. Duplicate of this sample, after standing $8\frac{1}{2}$ months, had the following gas content:

Carbon Dioxide	1.20%
Oxygen	.00%
Hydrogen	.60%
Nitrogen	98.20%

7. Can $\frac{1}{2}$ full, containing 300 cc $\frac{1}{4}$ % Citric Acid solution, sealed cold, processed one hour at 245° Fah.—after standing 42 hours had a gas content analyzing:

Carbon Dioxide	.40%
Oxygen	11.60%
Hydrogen	.00%
Nitrogen	88.00%

8. Duplicate of this sample, after standing $8\frac{1}{2}$ months, had a gas content analyzing:

Carbon Dioxide	.20%
Oxygen	.00%
Hydrogen	.30%
Nitrogen	99.50%

9. Can $\frac{1}{2}$ full, with 300 cc solution, $\frac{1}{4}$ % Citric Acid and $1\frac{1}{2}$% Sodium Chloride, sealed cold, processed one hour at 245° Fah.—after standing 42 hours, had the following gas content:

Carbon Dioxide	.40%
Oxygen	12.00%
Hydrogen	.00%
Nitrogen	87.60%

10. Duplicate of this sample, after standing $8\frac{1}{2}$ months, gave the following gas analysis:

Carbon Dioxide	.30%
Oxygen	.00%
Hydrogen	.80%
Nitrogen	98.90%

The following points in connection with these analyses are significant:

1st. Oxygen disappeared in all cans in the course of time, although the amount originally left in the cans was very excessive.

2nd. Oxygen disappeared in cans containing plain water, also in cans containing acid and acid and salt solutions.

3rd. Hydrogen was not found in any gas analysis until all of the Oxygen had disappeared, although steady acid corrosion had been going on.

If a stronger solution of Citric Acid had been used, much more Hydrogen would have been formed and probably the Oxygen would have disappeared more quickly.

The following analyses were obtained on commercial samples of food products:

Can, eighteen months old, containing Red Raspberries, furnished gas of the following analysis:

Carbon Dioxide	8.40%
Oxygen	.00%
Hydrogen	65.50%
Nitrogen	26.10%

Can, nine months old, containing Red Raspberries, furnished gas of the following analysis:

Carbon Dioxide	10.90%
Oxygen	.00%
Hydrogen	16.50%
Nitrogen	72.60%

Can, eighteen months old, containing Strawberries, furnished gas of the following analysis:

Carbon Dioxide	12.60%
Oxygen	.00%
Hydrogen	72.40%
Nitrogen	15.00%

Can, one year old, containing Strawberries, furnished gas of the following analysis:

Carbon Dioxide	13.20%
Oxygen	.00%
Hydrogen	27.20%
Nitrogen	69.60%

These containers were not properly protected by means of enamel, so that corrosion had been very excessive.

No analyses of gases from canned food containers have ever shown Hydrogen and Oxygen gas together, and inasmuch as Hydrogen Gas must necessarily be formed continuously from the beginning, when acid fruits are present, it would appear that the Hydrogen, under the conditions obtaining in a tin container, combines with Oxygen, and consequently cannot be found until all of the Oxygen has disappeared.

It would appear, therefore, that Oxygen disappears in tin food containers in at least the three following manners:

1st. By combining with the metals tin and iron, forming oxides.

2nd. By oxidizing tin or iron salts.

3rd. By combination with Nascent Hydrogen, when organic acids act on the metallic container.

It is also probable, in some instances, that Oxygen combines directly with the food product, during processing, particularly with such foods as Evaporated Milk, canned Salmon, Pork and Beans, etc. in which there are oily substances. In evaporated Milk and Pork and Beans, there is some caramelization which would also take up some Oxygen.

The analytical work reported in this paper was done by W. S. Sellars.

EGGS PRESERVED WITH SILICATE OF SODA

By J. M. Bartlett

Orono, Maine

For several years water glass or silicate of soda has been used for preserving eggs and has been endorsed by experiment Stations,* the Department of Agriculture** and some foreign scientists. With whom the idea originated the writer is unable to learn, but it was probably first used in Germany.† J. H. Thieriot in 1897 reports testing 20 methods of preserving eggs and obtaining the most satisfactory results with water glass. This method has not been used or recommended for commercial purposes, as cold storage is probably superior for that purpose; but for the family or home use, a supply of eggs can be carried from summer, when they are cheap, for use during the winter months when prices for fresh goods are beyond the means of ordinary incomes. Good eggs properly handled and preserved in water glass are much superior to most cold storage stock and for many purposes equal to fresh eggs.

To obtain the best results with this process it is necessary:

First, to have good fresh eggs.

Second, to have silicate of the right composition which does not contain free soda.

Third, the eggs should be kept completely covered with the solution in a galvanized iron or earthern vessel in a cool place.

The container should be covered to prevent evaporation. The composition of the silicate is a most important factor. Sodium oxide combines with silicon oxide in many different proportions and we find given in the chemical dictionary†† compounds containing from two parts Na_2O to one part of SiO_2 up to one

* Rhode Island Exp. Sta. Report 1900–1901 pp. 304.

* North Dakota Exp. Sta. Bulletin, 35, pp. 330–332.

** U. S. Dept. of Agriculture, Farmers' Bulletin 103.

† Experiment Station Record, Vol. 9, 1897–8, p. 981.

†† Nem's Handworterbuch der Chem., Vol. VI, p. 770.

part Na_2O to four parts SiO_2. The silicates containing such large proportions of Na_2O as the first mentioned would be too strongly alkaline for keeping eggs.* It has been found that eggs kept in a strongly alkaline solution absorbed some of the alkali and produce a jelly like condition of the whites. It is probable that the SiO_2 is not readily deposited from such solutions and the pores of the shell are not closed immediately, consequently some of the solution finds its way through to the interior and the property to which water glass owes its efficiency as a preservative is lost. The writer has obtained very satisfactory results with a silicate containing 24.2 parts of SiO_2 to 8.89 parts Na_2O made

	Six eggs weighed when put in solution	Six eggs weighed when taken out of solution
No. 1	57.18 gms.	57.12 gms.
No. 2	55.85 "	55.85 "
No. 3	62.40 "	62.35 "
No. 4	55.76 "	55.75 "
No. 5	56.67 "	56.62 "
No. 6	53.77 "	53.75 "
	341.63 "	341.44 "

into the glass by the dry process then dissolved by superheated steam and made up to a syrup testing 38 degrees B. One part of this syrup to nine parts of water makes a solution of about 0.045 specific gravity, in which fresh eggs readily sink and will remain submerged. Eggs kept in this way are of better flavor than cold storage stock. They never have the musty taste so often found in the storage goods and about the only difference between them and fresh eggs is a little lack of flavor. The shells are hermetically sealed and no bacteria can get through them, neither can oxygen, consequently if they contain any life when put in the solution it is very soon destroyed. Six fertile eggs put in a jar of water glass were kept in an incubator for 6 days at a temperature of 103 degrees F. and an examination

* Borntraeger (Oeslem. Chem. Ztg. 3, 1900, No. 12, p. 295.

at the end of that time was made, showing that the embryo had made no growth. There is very little change in the moisture content, and, unlike eggs in cold storage, the weight remains practically constant.

The writing of this paper and the limited investigation which is here given was suggested by a newspaper article which contained the statement that eggs preserved in water glass were unfit for food because they contained quite a quantity of soluble silica which if taken into the system was very dangerous and liable to cause coagulation of the blood. Notwithstanding the ridiculousness of the statement many people were alarmed and ceased to use eggs preserved in the silicate, and often inquiries were made to learn if any investigations had been made to determine if silica passed into the egg content. The work, therefore, was undertaken to determine principally whether eggs kept in water glass contained any more silica than fresh eggs. Some work was also done to determine if any marked changes take place in the nitrogen compounds. The most noticeable physical change in the eggs is a thinning out of the white which after the egg is kept 10 or 12 months does not coagulate so firmly as does that of a fresh egg, and the white appears much more watery.

The results of the investigation are given in the tables which follow. To separate the yolks from the whites completely, particularly in the preserved eggs, it was found necessary to boil the eggs before breaking them which, of course, caused some loss of moisture. The methods of analysis used in the experiments were those employed by the Bureau of Chemistry, U. S. Department of Agriculture,* in the work on cold storage eggs. The preserved eggs used were put down by the writer in a 10 per cent solution of 38 degrees B. water glass syrup, containing one part Na_2O to 2.7 parts SiO_2, in April 1911. They were, consequently, when examined about 11 months old. The other lot was put in the same kind of a silicate solution in February 1912 and examined in April, consequently were about two months in the solution.

* Bulletin No. 115, Bureau of Chemistry, U. S. Dept. of Agric.

WEIGHT OF EGGS, WHITES AND YOLKS AND LOSS IN BOILING

	Weight of Eggs Raw	Weight of Eggs Boiled	Weight of Shells	Weight of White	Weight of Yolks	Loss in Boiling	Total loss of moisture in boiling and shelling
	Grams	Grams	Grams	Grams	Grams	Per cent	Per cent
3 fresh eggs.....	171.272	166.838	17.93	88.51	59.301	2.59	3.81
3 w. g. eggs, one year.....	193.353	190.199	22.835	97.86	63.115	1.63	4.93
3 fresh eggs.....	175.102	170.304	18.071	98.683	52.738	2.74	3.20
3 w. g. eggs, 2 months.....	175.320	169.550	16.91	96.81	51.92	3.29	5.52

Partial Analysis of Fresh and Preserved Eggs

Wet Basis

	Moisture	Ash	Silica	Ether Extract (Petroleum)	Nitrogen present as				
					Total	Coagulated by boiling	Uncoagulated by boiling	Coagulated with salt and tannin	Uncoagulated
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Fresh Eggs									
White	87.40	0.785	0.005		1.78	1.585	0.195	0.115	0.08
Yolk	48.05	1.35	0.062	32.15	2.655	2.540	0.115	0.015	0.10
Preserved eggs. 11 months..									
White	85.15	0.70	0.006	0.02	2.05	1.675	0.375	0.365	0.11
Yolk	52.80	1.30	0.060	29.70	2.37	2.21	0.160	0.008	0.152
2 months in water glass....									
White	86.43	0.73	0.012	0.054	2.018	1.783	0.235	0.172	0.063
Yolk	50.25	1.47	0.040	30.68	2.53	2.405	0.125	0.015	0.110

Partial Analysis of Fresh and Preserved Eggs

Dry Basis

	Ash	Silica	Ether Extract (Petroleum)	Nitrogen present as				
				Total	Coagulated by boiling	Uncoagulated by boiling	Coagulated with salt and tannin	Uncoagulated
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Fresh Eggs.								
White.....	6.23	0.04		14.12	12.58	1.54	0.912	0.64
Yolk.....	2.64	0.119	62.98	5.20	4.98	0.225	0.029	0.195
Preserved eggs in water glass. 11 months.								
White.....	4.71	0.04	0.134	13.80	11.29	2.58	2.46	0.74
Yolk.....	2.46	0.113	56.25	4.49	4.19	0.30	0.15	0.288
Preserved eggs in water glass 2 months								
White.....	5.37	0.087	0.398	14.88	13.14	1.732	1.267	0.464
Yolk.....	2.93	0.080	61.04	5.04	4.81	0.23	0.03	0.22

In addition to the results given in the tables some work was done in coagulating the albumen of the white with different reagents. Three eggs of each kind were taken and the whites separated as completely as possible from the yolks in the raw condition. 10 gram samples of the whites were treated with acidulated water and boiled. The whites yielded copious flocculent precipitates which were thrown on tared filters, washed with hot water, dried and weighed.

The fresh eggs yielded dried albumen 10.0 per cent.

The 11 months old preserved eggs yielded dried albumen 10.28 per cent.

Samples treated with alcohol and allowed to stand several hours yielded:

Fresh eggs, dried albumen.....12.00 per cent.
Preserved eggs, dried albumen.....12.39 per cent.

Calculated from nitrogen content (n. x 6.25)

Fresh eggs	= 11.37 per cent.
Preserved eggs	= 11.62 per cent.

It is obvious from the figures here obtained and those given in the table that there is practically no difference in the total coaguable proteid matter of the fresh or preserved eggs. There seems to be a slight difference in the amount of nitrogen or protein coagulated by heat alone and is probably due to the presence of albumoses and peptones which are absent in fresh eggs but appear to develop as the esgg age.* This same change was noted in the case of cold storage eggs, and reported in a paper published by Dr. H. W. Wiley and others.†

Conclusions

1. Eggs packed in the right kind of water glass (silicate of soda) contain no more silica or other ash materials than fresh eggs.

2. The moisture content remains constant and a preserved egg weighs practically the same as when put in the solution.

3. The nutritive value as far as one can judge from the chemical analysis is the same as that of a fresh egg. The quality is superior to most cold storage eggs, as the pores of the shell are closed and no bad odors or flavors are absorbed.

* Allen's Commercial Organic Analyses, Vol. IV, p. 41.

† Bulletin 115, Bureau of Chemistry, U. S. Dept. of Agric., p. 32.

SOME OF THE RESULTS OF THE FOOD AND DRUGS ACT

W. D. Bigelow, Ph.D.

Chief of Division of Foods and Assistant Chief, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.

The complete study of this question would involve the principles governing the manufacture and sale of each particular kind of food and drugs. It would necessitate the discussion of the history and development of a large number of articles and of the varied influences which lead to the adoption and subsequent discontinuance of individual practices, in each. This would lead us into a maze of details which would require a considerable volume for their adequate presentation. In a paper of this scope, therefore, it is only possible to consider some of the general principles involved and a few of the fundamental changes that have resulted from the enforcement of the Act of June 30, 1906. At the time of the passage of the Food and Drugs Act, notwithstanding the creditable work that had been done by 27 States in the enforcement of their laws, labels on foods were very frequently so written as to deceive the consumer with respect to the character, value or origin of the product.

When the labels purported to give the weight of the product their statement was commonly exaggerated, sometimes being the gross weight of the product and package and sometimes having no relation to the weight of the product at all. Cans known technically as No. 1, 2, 3, etc., were sometimes designated as "1 pound," "2 pound," "3 pound," etc., though holding perhaps only two-thirds of that amount. Bottles of wine, oil and other products measuring five to a gallon,—sometimes a smaller amount,—were often labeled as quarts. Canned food and bottled goods are still sometimes referred to in grocers' lists and restaurants as "Pounds" and "Quarts," respectively, but the practice of designating them in that manner on the label and on the shipping case has been discontinued.

There are many difficulties encountered in marking the weight accurately upon a package of some kinds of food and it is probable that the misstatements that now exist on labels of food with respect to weight are often unintentional. The fraudulent statements which were prevalent five years ago have almost disappeared.

Closely related to misbranding with respect to weight is the practice in packing of canned food usually known as "slack filling." In such case the can was only partially filled with the food in question, the deficiency usually being made up with water to prevent the collapsing of the can. For instance, peas or beans were filled to within one-half inch, or possibly sometimes an inch, of the point to which they should be filled and then water or weak brine added to make up the proper volume. In packing cove oysters—in the extreme illustration of slack filling—only one and one-half ounces of oysters were sometimes placed in cans capable of holding over five ounces and the cans were then filled with brine. In tomatoes the cans were filled probably to within an inch of the top or sometimes only half or two-thirds full and the deficiency made up with water or perhaps weak brine. Steps were taken to correct this abuse and during the last year successful prosecutions have been maintained against packers of slack filled cans. The Department has announced publicly that this practice is fraudulent and it is believed that it has now been entirely discontinued.

Foods have been misbranded commonly with respect to the name of the place, (i. e. the country or region) in which they were produced or manufactured. This practice has obtained for two reasons: first, because of the desire of the packer in one locality to take advantage of a favorable reputation of another locality and so misbrand his goods as to the place of their manufacture; second, because of a certain glamor which a foreign name possesses for many consumers. As an illustration of the first class of abuses with respect to geographical name may be mentioned the packing of "Maine Sweet Corn" in Maryland, of "Michigan Apples" in Arkansas, of "California Canned Fruit" near the Atlantic Coast, of "Minnesota Flour" in the mills of Iowa and Missouri.

This form of misbranding gradually shades into the class where the misuse of a geographical name causes a false impression with respect to the material of which the food is made; for instance, the term "Vermont Sirup" or "Ohio Syrup" means maple sirup to the consumer because that is the only sirup made in Vermont and Ohio. These terms have been used frequently on the label of a cane sugar sirup colored and sometimes flavored in imitation of maple sirup. This form of misbranding has been corrected generally with reference to the staple articles of the United States.

Material progress has been made in correcting this form of misbranding in the case of foreign products and of foods manufactured in the United States in imitation of foreign products. Some of the brands of coffee which were formerly labeled "Mocha and Java," for instance, are now merely called "Coffee." Importers have been required to discontinue, on imported foods, the language of another country than that of their manufacture. Progress has been made in the correction of the label of imported wines which are commonly misbranded with respect to their character or class. Progress has also been made in the correction of the labels of certain products manufactured in the United States in imitation of foreign products, such as macaroni and tomato paste. Several kinds of cheese manufactured in imitation of well known foreign varieties are now labeled with the place of their manufacture. Rice grown in this country from Japan seed is labeled as grown in the United States. A product formerly called "Holland Rusk" with the label embellished with Dutch scenery is now labeled as made in Holland, Michigan, and the Dutch windmill has been removed from the label. This form of misbranding again merges into adulteration as, for instance, when cottonseed oil grown in the United States is placed in decorated tins so labeled as to represent the product to be an Italian olive oil. These practices still obtain to a certain extent, though to a much less degree than formerly.

One of the prevalent forms of misbranding is the use on the label of exaggerated claims regarding the strength and the nutritive value of the product. This form of misbranding is especially applicable to proprietary remedies but has also been practiced

largely with foods. Flavoring extracts were often labeled "Double Strength," or "Triple Strength," although the products so labeled were rarely beyond standard strength, and not infrequently were entirely fictitious. Breakfast foods and infant foods carried on their labels a glowing description of their miraculous nutritive value and sometimes curative properties. Cereal preparations of ordinary composition without any of the starch having been removed were sold under labels representing them to be diabetic foods. These practices have largely passed away as far as the labels are concerned. Unfortunately, the law does not reach posters and advertising matter sent through the mails and by such means fraudulent statements regarding the quality and nutritive value of some preparations is conveyed to the consumer. The labels themselves, however, have been greatly improved.

The addition to foods of substances of lower value to serve as a make weight and thus cheapen the food has largely been discontinued. At the time of the passage of the Food and Drugs Act such practices had been made away with in a number of the States but in other States and indeed in interstate commerce they were still quite prevalent. As illustrations of this practice may be cited rye flour and buckwheat flour, both of which contained a substantial amount of wheat flour; spices which were commonly loaded with cereal preparations, ground olive stones, cocoanut shells, etc., a line of preparations being manufactured and sold for the purpose of adulterating spices and pepper shells and olive stones being imported into this country for that purpose.

At the time of the passage of the Food and Drugs Act there was little pure maple syrup manufactured commercially and sold in interstate commerce. Immediately after the law went into effect there was practically no maple syrup to be had but brands of so-called maple syrup which had formerly borne on the label an offer of a large reward to anyone who would prove the presence of any adulterant appeared under a new label, declaring the contents of the package to be a mixture of maple syrup and cane sugar syrup.

Cider vinegar was commonly diluted with water to reduce its strength in acetic acid to the desired percentage and since this dilution brought the solids content down to a lower figure than

that in commonly accepted standards a quantity of boiled cider was added. It only remained to add a larger quantity of boiled cider and, of water and then to strengthen with distilled vinegar to obtain much larger yields and this practice merged gradually into the preparation of an entirely fictitious product manufactured from distilled vinegar, with color added and solids in the form of boiled cider. The detection of practices of this kind by analytical means offered many difficult problems which have been partly solved and maple sirup and cider vinegar may be cited as types of a large number of products which are now sold in the pure state to a very much larger degree than was true at the time of the passage of the Food and Drugs Act.

The two classes of substances relied on chiefly by the manufacturer in the preparation of fictitious products are colors and flavors. It was a difficult matter to handle either of these classes of substances in such a way as to imitate a natural food. The manufacturer who uses them is likely to go to extremes and the fictitious products he puts on the market are frequently of a hue that is scarcely to te found in nature, whereas the flavors are also commonly in excess. The improvement in natural products that has attended the work of the last five years, accompanied by the better information of the public regarding such matters, has resulted in a growing aversion for fictitious colors and flavors and many lines of products which were formerly in demand are now regarded by the public with disfavor. Moreover the wholesomeness of the colors employed has been considered. The Department has authorized the use in foods of a list of 7 coal tar colors and these must be manufactured in such a way as to be free from arsenic and other deleterious substances. Manufacturers have to a large extent complied with this regulation.

To a much greater extent than ever before manufacturers are giving attention to the question of the wholesomeness of substances used in the preparation of foods. Formerly this was not the case. When it was desired to begin the use of a preparation in the manufacture of foods the ordinary article of commerce was frequently employed without any thought of its possible injurious properties. When acid phosphate was employed, for instance, in the preparation of a food or drug the acid phosphate of com-

merce was used and it was not known that it contained a considerable amount of arsenic. Notwithstanding the fact that lead pipes have been known for generations to be improper for conducting water for household purposes, they were employed for tartaric and citric acid which were intended to be used as foods and a relatively large amount of lead thus found its way into the product placed on the market for ordinary consumption.

When a confectioner desired to give a gloss to some of his wares it occured to him that the product used by the painter would meet his requirements and he took ordinary shellac without considering whether the lac itself was injurious to health and without thinking of the fact that the shellac of commerce contains a considerable amount of arsenic. We even found a large shipper of green coffee who desiring to polish his wares and give them a faint yellowish shade, used the first yellow powder which came to his attention and this happened to be chromate of lead. When it was desired to prolong the life of certain foods and at the same time make unnecessary the care in handling which would otherwise be necessary among the preservatives suggested and largely employed are some substances whose toxicity was universally admitted; e.g.—formaldehyde and ammonium fluorid.

The important point is not that some practices of this nature have been corrected and others are being corrected at this time, but that there is a rapidly growing tendency on the part of manufacturers when considering the use of a new or unusual substance or preparation in the manufacture of food to consider whether it is injurious to health, either because of its nature or composition or because of certain impurities and whether for any reason its addition to food is objectionable.

The removal of the manufacture of prepared foods from the home to the factory has made great changes in our civilization and made necessary precautions which were before unthought of. One of the most prominent characteristics of civilization is the increased emphasis placed on cleanliness and sanitary conditions. It is only this fact which has made possible in the home during the last century the preparation of many of the domestic preserved foods which are now most prevalent. The manufacture of these foods in the factory, however, has not been confined to men who

were qualified to enforce sanitary conditions such as are necessary for the successful preparation of many articles of food. The result has been that we have had placed on the market on the one hand preparations in a more or less advanced state of decay and on the other hand substances contaminated with pathogenic organisms. Thus there have been cases of contaminated water being bottled and sold as spring water and being used for the preparation of soft drinks and for serving from soda fountains.

Tomato catsup has been prepared from the peelings and cores of unwashed tomatoes, including a considerable part of rotting material, and by a process and amid surroundings which caused additional decomposition to take place during the course of manufacture. Ripe olives and figs were often imported into the United States in a wormy and decomposed condition. So little attention was given to the matter that it was the custom of railroads to sell unclaimed food products resulting from wrecks to the highest bidder with a knowledge that they would be placed on the market indiscriminately.

Badly contaminated water has been used for cleansing milk cans in dairies and together with contaminated ice has not infrequently been added to the milk. The sanitary condition of dairy stables has frequently been bad. Eggs so far advanced in decomposition that they would not be used by a housekeeper have been broken in large quantities and placed on the market either dried or frozen. Oysters and clams have been taken from contaminated water and placed on the market with the inevitable result of spreading typhoid fever.

These sanitary problems offer difficulties which cannot be overcome in a day but in all of them material progress has been made. A number of the States, realizing the importance of sanitary requirements, have enacted special sanitary laws whose enforcement has done much to improve the conditions formerly existing. Of still greater importance, however, is the fact that manufacturers as a whole have become interested in the desiderata of a food manufacturing establishment from a sanitary standpoint and the changes resulting in the cleanliness of their establishments, as well as in their utensils and the character of the raw material they employ, are among the most satisfactory

results of the recent enforcement of food legislation. Whereas formerly only rule of thumb methods were employed we now meet chemists, bacteriologists and microscopists in many general food manufacturing establishments. It is frequently made the duty of some special officer to study and be responsible for the sanitary condition of the factory. The health of the employees is considered with reference to the influence it may have upon the food. Cleanliness is more frequently required, as well as uniforms or special factory clothes, and in some establishments manicurists are employed.

The number of prosecutions that have been successfully maintained for the violation of the law is of minor importance compared with this change in the attitude of the manufacturers.

SUR L'ANALYSE DU PHOSPHORE DANS LES CENDRES DU LAIT

M. le Dr. Bordas

College de France, Paris, France

L'expérience nous a démontré que l'acide phosphorique existant à l'état de phosphates dans les cendres d'un lait correspond à la totalité du Ph contenu dans ce liquide, c'est-à-dire au Ph minéral et au Ph organique: lécithine, nucléine, etc. . . . Cette particularité est très importante à connaitre pour éviter certaines erreurs d'appréciation sur la valeur alimentaire du lait.

Il s'ensuit donc, pour le cas particulier du lait de vache, lorsqu'on fait les cendres de ce liquide on ne provoque aucune disparition de phosphore par l'action du charbon sur les phosphates, et la matière grasse n'entraine aucune partie du phosphore à l'état de combinaison volatile. Il est inutile d'ajouter des sels de chaux, de baryte, de magnésie, etc., comme le préconisent plusieurs auteurs, pour éviter des pertes en phosphore par calcination.

Le phosphore total d'un lait peut donc être dosé directement sur ses cendres. D'autre part, en précipitant le lait par l'acide trichloracétique on détermine le Ph minéral dans le lactoserum et le Ph organique dans le coagulum.

L'ACIDITE ORIGINELLE DU LAIT

M. le Dr. Bordas

College de France, Paris, France

Les auteurs ne sont pas tous d'accord sur la réaction à attribuer au lait, pour les uns ce liquide, à l'état frais, serait acide, pour les autres, il serait amphotère, c'est-à-dire possèderait une réaction alcaline et une réaction acide.

En étudiant cette question nous avons constaté que ces divergences d'opinions résidaient uniquement dans l'emploi d'indicateurs qui ne répondaient pas aux conditions expérimentales.

Nous avons établi que la phtaléine du phénol est l'indicateur de choix pour étudier la réaction du lait. Lorsque ce liquide est précipité par notre réactif alcool 65° acétique à 1-1000 nous constatons, en tenant compte de l'acidité du réactif, que l'acidité totale d'un lait frais se retrouve dans le coagulum et qu'elle est due exclusivement à la caséine libre.

L'expérience nous a démontré également qu'il existe dans un lait frais aucun acide libre, lactique, citrique, ni aucun sel à fonction acide, que l'augmentation de l'acidité d'un lait provient tout d'abord de la caséine déplacée, de sa combinaison calcique, par l'action de l'acide lactique formé aux dépens du lactose et que l'acidité lactique n'apparait ensuite que lorsque cet acide a réagi sur les sels de chaux du lait.

DE L'ACTION DU LAIT SUR CERTAINS RÉACTIFS

M. le Dr. Bordas
College de France, Paris, France

Pour expliquer les phénomènes péroxydasiques obtenus au sein d'un liquide on s'appuie en général sur l'existence de substances diastasiques que certain considère comme une individualité définie. Or, jusqu'ici, il n'a pas été possible d'isoler à l'état de pureté les matières diastasiques actives et toujours nous les retrovons à côté d'éléments minéraux. Il existe donc une relation étroite entre tous ces éléments et leur ensemble constitue un système péroxydasique que nous retrouvons dans l'étude des diastases du lait.

Le but que nous poursuivons consiste à rechercher le mécanisme qui préside aux réactions colorées obtenues dans le lait avec certains réactifs.

Nous allons nous occuper, en particulier de l'action de la paraphénylènediamine sur le lait, ce corps formant le réactif le plus sensible pour la recherche des péroxydases du lait.

Dans cette étude nous avons été amenés à reproduire artificiellement des phénomènes péroxydasiques à l'aide de substances prises souvent en dehors des matériaux existant dans le lait.

On sait que la paraphénylènediamine par oxydation forme de la quinone. Cette oxydation se produit déja en laissant exposer à l'air une solution aqueuse de cette base qui devient *plus ou moins brune* suivant le temps d'exposition. On arrive également à ce résultat par l'action d'un courant d'O ou par la décomposition de l'H^2O^2 au sein d'une solution aqueuse de cette diamine, mais on obtient aucune coloration bleue comme celle qui se produit dans un lait frais additionné d'H^2O^2 et de paraphénylènediamine. Cette coloration n'est donc pas le résultat d'une simple oxydation, il est en effet nécessaire de faire intervenir une autre cause pour expliquer la coloration bleue.

Nous avons constaté qu'elle était due à l'action d'un produit intermédiaire entre la paraphénylènediamine et la quinone sur les sels de chaux.

Il nous suffit par exemple, de verser une goutte d'une solution de paraphénylènediamine sur un bâton de craie pour obtenir immédiatement une coloration bleue.

Ceci nous conduit à obtenir directement cette coloration avec certains sels de chaux en même temps que ces sels nous servent de catalyseurs de H^2O^2 pour l'oxydation de la paraphénylènediamine.

Prenons, en effet, du citrate de chaux on du phosphate tricalcique purs et secs, ajoutons 2 ou 3 gouttes d' H^2O^2 et mélangeons, si on ajoute ensuite une goutte ou deux d'une solution fraiche de paraphénylènediamine à 2% on obtient immédiatement la coloration bleue en question qui se fixe sur le sel de chaux. Si avant d'ajouter le réactif on délaye dans l'eau les sels de chaux insolubles et oxygénés on constate encore la coloration bleue qui reste toujours fixée sur la chaux.

Cette réaction se divise donc en deux phrases:

1°—Oxydation de la base par un phénomène catalytique.

2°—Coloration bleue produite par la chaux du sel sur le réactif oxyde.

Nous avons toujours obtenu ces résultats avec des poudres sèches imprégnées d'H^2O^2 et renfermant de la chaux, et toutes les fois que la chaux n'était plus en présence la réaction était négative.

Les expériences suivantes sont en effet très concluantes.

Si dans deux petites capsules on place dans l'une de la pierre ponce ordinaire et dans l'autre la même pierre ponce traitée par l'eau régale, lavée à l'eau et sèchée à 100°, puis qu'on répète l'expérience précédente, on constate que les grains de pierre ponce ordinaire sont seuls colorés. Les mêmes résultats sont également obtenus avec de la caséine privée ou non de sa chaux comme nous l'avons signalé dans un travail antérieur. Nous ferons remarquer en outre, que cette coloration bleue ne se produit que dans un milieu très légèrement acide.

En résumé, nous pouvons admettre, d'après ces expériences,

que l'oxydation de la paraphénylènediamine produit un laque bleu-indigo en présence d'un sel de chaux.

Voyons maintenant ce qui se passe lorsqu'on recherche les péroxydases du lait au moyen de la paraphénylenediamine.

D'après les théories actuelles il existerait dans le lait frais-des diastases capables de décomposer l'H^2O^2 et l'oxygène mis en liberté provoquerait la coloration bleu-indigo de la paraphénylènediamine, d'autre part, on sait qu'un lait porté à 80° perd la propriété de décomposer l'eau oxygénée.

La modification apportée par la chaleur dans un lait frais est due à la coagulation d'une partie de la matière protéique qui empêche la décomposition de l'H^2O^2 ajoutée au lait, mais nous sommes parvenus à isoler néanmoins le catalyseur d'un lait bouilli au moyen de la centrifugation comme nous l'avons démontré antérieurement, c'est-à-dire, en recueillant le dépôt formé au fond du tube du centrifugeur et la crème qui surnage. Ces deux parties du lait bouilli ou stérilisé sont capables de décomposer l'H^2O^2 et oxyder la paraphénylènediamine donnant la coloration bleue en présence de la chaux.

Une expérience nouvelle plus concluante encore nous a permis d'obtenir une réaction positive avec la paraphénylènediamine sur le lait entier chauffé à plus de 80° mais homogénéisé à l'aide d'une machine pulvérisant le lait à une pression de 200 atmosphères. Cette opération ayant pour effet de donner à toutes les molécules, des corps insolubles contenues dans le lait une même dimension. On rétablit ainsi, dans une certaine mesure, l'état colloïdal primitif du lait et son catalyseur a pu agir à nouveau sur l'H^2O^2 et donner en présence de la paraphénylènediamine la coloration bleue.

Nous écartons dans cette expérience comme dans toutes celles que nous avons faites sur les laits bouillis les causes d'erreur dues à la présence de bactéries ou de muscédinées qui peuvent exister lorsqu'on opère avec des laits altérés.

Nous avons constaté que la réaction avec du lait homogénéisé est moins intense qu'avec du lait frais mais il n'en reste pas moins établi que nous pouvons redonner par un procédé mécanique à du lait chauffé à plus de 80° son pouvoir péroxydasique.

Nous avons encore démontré avec ces laits que la coloration

bleue du réactif employé est bien due à la présence des sels de chaux du lait. En effet, en prenant un lait fixé et stérilisé, nous pouvons lui rendre son maximum d'action en introduisant dans ce lait un catalyseur artificiel, soit une poudre pulvérulente insoluble sans action chimique comme la Sio^2 pure par exemple, soit des traces d'une solution d'oxalate de fer. Dans ces conditions la coloration bleue avec la paraphénylènediamine devient très intense.

Toutes ces expériences démontrent bien que les réactions négatives avec le réactif à la paraphénylènediamine dans un lait chauffé à 80° ne sont dues qu'à un changement d'état physique du lait et que les péroxydases ou les catalases qui ont été signalées, et qu'aucun auteur n'a pu isoler à l'état de pureté doivent être considérées jusqu'à présent comme des combinaisons organo-métalliques jouant un rôle chimique et non biologique.

A CHEMICAL INVESTIGATION OF ASIATIC RICE

By Allerton S. Cushman and H. C. Fuller

Institute of Industrial Research, Washington, D. C.

Introduction.

The following paper contains a description and the results of a complete chemical investigation of twenty-seven samples of Asiatic rice, which was recently carried out at the instance of the Siamese Government. The samples were collected in the open market at Singapore and Shanghai and no effort was made to prepare them in any way differently from those rices which are ordinarily exposed for sale in the Asiatic market. The relation of an exclusive rice diet on the etiology of beri-beri disease has been much discussed for a number of years past. This paper does not pretend to decide this controversy but is offered as a contribution to the general knowledge of the chemical constitution of rice. As far as the authors are aware the results on the phosphate content of eastern rices is the most complete as yet published.

Description of Samples.

The samples reached the Institute on October 30th, 1911, and the box containing them was opened on October 31st. The samples were contained in twenty-seven 10 pound cotton bags numbered serially 1 to 27. No other distinguishing marks or information was found.

The cotton bags were found to be frail and rotten and in some cases were broken through, so that the contents had partially escaped. All the samples contained living weevils, and a few worms and beetles were also found. The condition of the samples made it necessary to hand pick them to remove insects. They were then immediately packed in glass bottles, stoppered and labeled.

The appearance of the samples indicated that they represented a medium grade of white or milled rices. On the trip from the

Far East the samples had evidently suffered desiccation with the result that some of the grains had become abraded and broken. As it was not believed, however, that the grain had suffered in such a way as to affect the chemical analysis except in regard to moisture content and the weight per 100 grains, it was decided to be unnecessary to delay the investigation by awaiting a new importation of samples from the Far East.

The Analytical Work.

The analytical work was carried out by the methods recommended by the Association of Official Agricultural Chemists of the United States, and comprised the following elements usually sought: Moisture, Ash, Proteids, Ether Extract (mainly Fat), Fibre, Starch and other Carbohydrates, Weight per 100 Grains.

The above determinations have usually been accounted sufficient to fix the nutrition value of a given cereal. In view, however, of a recently published claim that milled rices are deficient in organically combined phosphorous, phosphate determinations were carried out on each sample. The results have been carefully checked and may be taken as accurate for the samples worked on.

Tabulation of Results.

The results of the analytical work on the twenty-seven samples submitted are given in Table I, with the exception of the phosphate contents which are tabulated separately in Table III. Table II gives the results of analysis of two fresh samples of South Carolina (U. S. A.) rices bought at a prominent grocery house in Washington, D. C. These samples are denominated Numbers 29 and 30. Sample 29 is the ordinary very white large grained rice as sold in the United States at about ten cents a pound. Sample 30 was sold for a slightly higher price and purported to be a "natural uncoated special pure rice." Table III gives the phosphate content of all samples, reported as phosphoric anhydride, P_2O_5. In Appendix A are given the results of an examination of various rices exhibited at the World's Columbian Exhibition, at Chicago, in 1893, the analyses made by

the Division of Chemistry, U. S. Department of Agriculture. Appendix A is preceded by an extract from Bulletin No. 13, and is followed by a summing up of the results.

TABLE I

RESULTS OF ANALYSIS OF TWENTY-SEVEN SAMPLES OF RICE SUBMITTED TO THE INSTITUTE OF INDUSTRIAL RESEARCH BY THE SIAMESE LEGATION, WASHINGTON, D. C.

Sample No.	Weight of 100 Grains	Moisture	Ash	Ether Extract	Crude Fibre	Proteids	Starch and Carbohydrates
1	1.565 grams	11.02%	0.46%	0.31%	0.40%	8.13%	79.68%
2	1.39 "	10.99%	0.51%	0.29%	0.60%	8.25%	79.36%
3	1.181 "	11.11%	0.56%	0.20%	0.29%	7.38%	80.46%
4	1.036 "	10.82%	0.46%	0.15%	0.20%	8.44%	79.93%
5	1.708 "	11.54%	0.40%	0.13%	0.82%	8.44%	78.67%
6	1.651 "	10.51%	0.49%	0.28%	0.83%	7.56%	80.33%
7	1.498 "	11.14%	0.50%	0.20%	0.72%	7.81%	79.63%
8	1.244 "	11.31%	0.48%	0.15%	0.47%	7.75%	79.84%
9	1.481 "	11.10%	0.55%	0.68%	0.66%	8.31%	78.70%
10	1.409 "	11.30%	0.41%	0.63%	0.43%	7.81%	79.42%
11	1.329 "	10.60%	0.49%	0.20%	0.21%	7.63%	80.87%
12	1.725 "	11.28%	0.47%	0.31%	0.27%	7.56%	80.11%
13	1.723 "	10.45%	0.45%	0.17%	0.60%	8.06%	80.23%
14	1.541 "	10.94%	0.44%	0.53%	0.76%	7.56%	79.77%
15	1.141 "	10.44%	0.54%	0.10%	0.31%	7.81%	80.80%
16		11.08%	0.85%	0.28%	0.44%	8.25%	79.10%
17	0.958 "	10.51%	0.74%	0.12%	0.16%	7.81%	80.66%
18	0.892 "	10.49%	0.60%	0.30%	0.32%	8.00%	80.29%
19	0.788 "	9.99%	0.48%	0.94%	0.33%	8.06%	80.20%
20		10.06%	0.55%	0.71%	0.51%	8.13%	80.04%
21	1.238 "	9.21%	1.23%	0.80%	0.77%	8.44%	79.55%
22	1.175 "	9.19%	0.72%	0.87%	0.56%	8.94%	79.72%
23	1.533 "	9.32%	0.57%	0.52%	0.45%	8.75%	80.39%
24	1.179 "	9.55%	0.77%	0.91%	0.47%	8.38%	79.92%
25	1.429 "	10.37%	0.58%	0.16%	0.23%	8.38%	80.28%
26	1.413 "	10.04%	0.72%	0.59%	0.45%	7.63%	80.57%
27	1.581 "	10.81%	0.51%	0.44%	0.31%	8.63%	79.30%

TABLE II

RESULT OF ANALYSIS OF TWO SAMPLES OF SOUTH CAROLINA RICE

Sample No.	Weight of 100 grains	Moisture	Ash	Ether Extract	Crude Fibre	Proteids	Starch and Carbohydrates
29	2.241 grams	10.23%	0.47%	0.42%	0.29%	9.00%	79.59%
30	2.238 "	9.01%	0.37%	0.21%	0.36%	8.13%	81.92%

TABLE III

RESULTS OF PHOSPHATE DETERMINATIONS ON TWENTY-SEVEN SAMPLES OF RICE SUBMITTED TO THE INSTITUTE OF INDUSTRIAL RESEARCH BY THE SIAMESE LEGATION, WASHINGTON, D. C.

Sample No.	$\%P_2O_5$	Sample No.	$\%P_2O_5$	Sample No.	$\%P_2O_5$
1	0.22	10	0.31	19	0.31
2	0.39	11	0.32	20	0.30
3	0.30	12	0.23	21	0.41
4	0.20	13	0.21	22	0.39
5	0.28	14	0.21	23	0.42
6	0.26	15	0.30	24	0.58
7	0.31	16	0.49	25	0.24
8	0.26	17	0.35	26	0.22
9	0.30	18	0.35	27	0.34
South Carolina rice.....		29	0.29		
		30	0.24		

Interpretation of Results.

A careful inspection of the results shows, that all of the analyses of the samples submitted compare favorably in respect to nutrition value with the samples given under the World's Fair report which includes typical rice analyses as quoted by various authorities (see Appendix A). The results also for the most part compare well with the analyses of the South Carolina rices given in Table II. The phosphorous content of the imported

samples (Table III) shows considerable variation; in some cases it corresponds to the average for milled white rice which is reported to be about 0.25%; in other cases it is as high as is usually shown in rices treated by the parboiling process. It would appear that the white rices as represented in the twenty-seven imported samples show on the average as high a nutrition value as the white rices from other sources. The moisture content and weight per 100 grains is somewhat low in the imported samples, for the reason stated above.

Interpretation of the Analytical Results in Relation to the Etiology of Beri-Beri.

It has recently been claimed by Doctors Fraser and Stanton of the Institute for Medical Research, Kuala Lumpor, that the low phosphorous content of white milled rices is a predisposing cause of beri-beri. (See "The Lancet" London) Vol. 176, p. 451 (1909). It is further stated by Doctors Fraser and Stanton that: "From epidemilogical considerations and from experimental evidence it appears that Siam rice is considerably more potent in its beri-beri producing powers than Rangoon rice."

Opposed to the conclusions of Doctors Fraser and Stanton stands the opinion of Dr. Hamilton Wright, former Director of the Institute for Medical Research, Federated Malay States, an eminent investigator of the Etiology and Pathology of Beri-beri. Dr. Wright's published opinion, based on years of study and clinical experimentation is quoted below:

(An inquiry into the Etiology and Pathology of Beri-beri. Hamilton Wright, M. D., Studies from Institute for Medical Research, Federated Malay States, Vol. 2, No. 1, p. 58 (363).

"The theory of the causation of beri-beri that fits the above facts and all others observed in British Malaya is that beri-beri is due to a specific organism which gains entrance to the body via the mouth, that it develops and produces a toxin chiefly in the pyloric end of the stomach and duodenum, and that the toxin, being absorbed, acts atrophically on the peripheral terminations of the afferent and efferent neurones. Further, that the specific organism escapes in the fæces and lodges in confined places through accident or the careless personal habits of those

affected by the disorder, and that in the presence of congenial meteorological, climatic and artificial conditions of close association from overcrowding, the organism becomes virulent and, gaining entrance to the healthy body in food, etc., contaminated by it, gives rise to an attack of the disease. The fact that the germ remains so closely focal can, I think, be explained by its being at once destroyed by the action of direct sunlight or that the presence of CO_2 or some other gas is necessary for its virile development. It seems from my observations here that the active stage of the organism in the body is between three and four weeks. I base this estimation on the facts that the preliminary feeling of oppression in the epigastrium ceases at the end of about three weeks, and that it is rare to find the lesion of the gastric and intestinal mucose in cases of only six weeks' standing."

Conclusion.

As far as the results of analysis can be interpreted in the light of the information at hand, there would appear to be no reason why the white milled rices from one section of the world should be held more responsible for mal-nutrition than similar rices from other sections.

APPENDIX A.

EXTRACT FROM BULLETIN NO. 13, U. S. DEPARTMENT OF AGRICULTURE. DIVISION OF CHEMISTRY

Foods and Food Adulterants. Investigations made under direction of H. W. WILEY, Chief Chemist. Part 9. Cereals and Cereal Products, Washington, D. C., 1898.

Rice may reach the analyst in three different states, viz.: unhulled, hulled, and polished. He may also have occasion to examine the broken fragments used in polishing and hulling, the waste in manufacturing rice bran and other products. The most important of these products in the present connection is the polished rice as it is found in commerce, ready for preparation as food. Rice is a cereal in which the starchy matters predominate, and in which there is a marked deficiency of proteids and

oils as compared with other standard cereals. The composition of rice, as determined by the analysis of samples exhibited at the World's Columbian Exposition, and by standard authorities, is best shown in the table of maxima, minima, and means, as in the case of the other cereals which have been mentioned. In the following table the items marked I, II, and III, represent data obtained at the World's Columbian Exposition, while the means of all the samples there analyzed are given in another part of the table.

Table of Maxima, Minima, and Means of Constituents of Rice

Kinds and Nos. of samples	Wt. of 100 kernels	Moisture	Proteids	Ether extract	Crude fiber	Ash	Carbohydrates, excluding fiber
	Grams	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1. Rice in the hull (foreign):							
Maxima	a3.250	b11.52	b8.40	b2.04	b11.47	a4.66	a65.70
Minima	b2.842	a9.03	a8.23	a1.44	b9.45	b3.26	a65.01
Means	2.979	9.88	8.32	1.71	10.62	4.12	65.35
2. Unpolished rice (foreign):							
Maxima	c2.826	c12.57	c10.50	c2.26	c1.00	c1.22	c77.34
Minima	c2.260	c10.92	c7.27	c1.62	c0.87	c1.04	c73.35
Means	2.466	11.88	8.02	1.96	0.93	1.15	76.05
3. Polished rice (foreign):							
Maxima	b2.633	b13.15	b10.33	c0.54	a0.56	a0.65	c81.66
Minima	a1.560	c11.82	c5.42	c0.04	a0.27	a0.28	b75.62
Means	2.132	12.34	7.18	0.26	0.40	0.46	79.36
Mean composition of polished rice, etc., as given by Jenkins and Winton.							
Polished rice (10 analyses)	.....	12.40	7.40	0.40	0.20	0.40	79.20
Rice bran (5 analyses)	.....	9.70	12.10	10.90	9.50	10.00	49.90
Rice hulls (3 analyses)	.....	8.20	3.60	0.70	35.70	13.20	38.60
Rice polished (4 analyses)	.....	10.00	11.70	7.30	6.30	6.70	58.00

a Guatemala. b Johore. c Japan.

Table of Maxima, Minima, and Means of Constituents of Rice.—*Continued*

Kinds and Nos. of samples	Wt. of 100 kernels	Moisture	Proteids	Ether extract	Crude fiber	Ash	Cabohydrates, excluding fiber
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Mean composition of rice, etc., as given by Konig.							
Unhulled rice (3 analyses)	.....	11.99	6.48	1.65	6.48	3.33	70.07
Hulled rice (41 analyses)	.....	12.58	6.73	1.88	1.53	0.82	76.46
Polished rice (9 analyses)	.....	12.52	7.52	0.84	0.48	0.64	78.00
Means of World Fair samples.							
Unhulled rice (4 analyses)	2.929	10.28	7.95	1.65	10.42	4.09	65.60
Unpolished rice (6 analyses)	2.466	11.88	8.02	1.96	0.93	1.15	76.05
Polished rice (14 analyses)	2.132	12.34	7.18	0.26	0.40	0.46	79.36

The mean composition of the different classes of rice as shown by the analyses of the World's Fair samples is almost the same as that shown by the work of other analysts collated as indicated above. A typical unhulled rice has about the following composition:

Weight of 100 kernels, grams	3.00	Crude fiber, per cent	9.00
Moisture, per cent	10.50	Ash, per cent	4.00
Proteids, per cent	7.50	Carbohydrates, other than crude fiber, per cent	67.40
Ether extract, per cent	1.60		

A typical hulled rice, but unpolished, has about the following compositions:

Weight of 100 kernels, grams	2.50	Crude fiber, per cent	1.00
Moisture, per cent	12.00	Ash, per cent	1.00
Proteids, per cent	8.00	Carbohydrates, other than crude fiber, per cent	76.00
Ether extract, per cent	2.00		

A typical polished rice has a composition represented by the following numbers:

Weight of 100 kernels, grams	2.20	Crude fiber, per cent	0.40
Moisture, per cent	12.40	Ash, per cent	0.50
Proteids, per cent	7.50	Carbohydrates, other than crude fiber, per cent	78.80
Ether extract, per cent	0.40		

SULLA MATURAZIONE DEL FORMAGGIO PECORINO

Prof. Dr. E. De' Conno

Della R. Università, Napoli, Italy

Il pecorino è un formaggio grasso costituito di sostanze azotate e grasse; si ottiene dal latte di pecora, che ne può dare fino al 22 % (fresco) = la cagliata si cuoce e poi si foggia in pani cilindrici che vengono salati e si fanno stagionare almeno per nove mesi.

La sua pasta è bianco-gialliccia, omogenea o con piccoli e scarsi occhi = ha sapore ed odore piccanti, come i latticini pecorini in genere e tanto più pronunziati quanto più il formaggio è stagionato.

Il costituente principale del formaggio è la caseina, la quale, durante il periodo della maturazione, per effetto di speciali fermentazioni, origina materie albuminoidi solubili, ammidi prodotti ammoniacali, altre sostanze di natura ancora non ben definita e forse anche sostanze grasse.

Il formaggio contiene, oltre la caseina, acqua, sostanze grasse, lattosio, sali minerali (NaCl) in proporzioni molto variabili a secondo della provenienza dei modi di fabbricazione e dell'età.

Lo studio delle trasformazioni che il formaggio subisce quando è abbandonato all'azione delle diastasi e dei microrganismi, i quali fanno ad esso subire un cambiamento completo, fornisce uno dei più importanti capitoli della chimica del formaggio.

Questo prodotto durante la maturazione subisce a poco a poco diverse modificazioni. La massa caseosa prende un aspetto untuoso, nello stesso tempo che si sviluppano l'odore e il sapore che caratterizzano il formaggio maturo, nel quale l'analisi indica piccole quantità di ammoniaca, di acidi grassi e di leucina.

In principio, nel formaggio fresco vi sono necessariamente gli elementi del latte = caseina, burro ed anche del lattosio che la premitura non ha completamente eliminato.

Era interessante ricercare ciò che divengono questi principi durante la fermentazione casica. La perdita di peso che la materia subisce in seguito a questa fermentazione, perfettamente

constatata dall'esperienza è dovuta alla distruzione totale o parziale dell'uno o dell'altro di questi principi? Quali sono quelli che resistono, quali quelli che spariscono? La materia grassa è realmente aumentata nell'invecchiamento del formaggio?

In conseguenza di uno studio sul formaggio di Roquefort il Blondeau (1) affermò che nella maturazione del formaggio si formavano-dei principî grassi a spesa della caseina, la qual cosa fu poi confermata da Kemmerick (2) e da Fleischer (3) nonchè da Musso e Menozzi (4), i quali credono appunto che la formazione del grasso nelle stracchino abbia origine indirettamente dalla caseina.

Il Brassier (5) intanto poco dopo del Blondeau, in uno studio sulla trasformazione della caseina nella maturazione dello stesso formaggio Roquefort, criticando fortemente le esperienze del Blondeau, che egli dice "ben lontane d'essere al riparo di ogni critica" afferma che non vi è formazione di grassi a spesè della caseina; e gli studi di Manetti e Musso sul parmigiano confermano questa opinione. Anche le ricerche del Kellner (6) sulla quantità di burro in confronto di quella dell'acido fosforiéo e della calce confermano la stessa cosa, la quale provano pure le determinazioni di E. Schulze ed U. Weidmann (7).

Tali quistioni io ho voluto riesaminare analizzando il formaggio in diverse epoche, cominciando dal momento in cui fu coagulato e pressato.

Ho esaminato il pecorino da un punto di vista chimico, seguendo le trasformazioni che la caseina subisce dal momento della coagulazione fino a quello nel quale il formaggio maturo è messo in commercio per la consumazione.

Si comprende facilmente che la maggiore difficoltà consisteva nel preparare un formaggio fresco di costituzione sufficientemente omogenea ed ecco come ho proceduto:

Del latte di pecora fu lasciato per 24 h. in cantina, indi, separatane la crema, fu fatto agire il presame a 35°. Dopo la presa del

(1) Ann. de Chim. et de phys (4), I. 208.
(2) Pflüger's Archiv. f. d. gesammte Physiologie (1869), 409.
(3) Virchow's Archiv. f. pathol. Anatomie u. phys. (1871), LI. 40.
(4) Le stazioni sperimentali agrarie italiane (1877), VI. 201.
(5) Ann. de Chim. et de phys (4), V, 270; C. (1865), 888.
(6) Landw. Versuchsst (1880), XXV. 39.
(7) Landw. Jahrbücher (1882), XI. 587.

caglio fu favorito lo scolamento del siero mettendo a sgocciolare il siero sopra una tela e sottomettendolo poi ad una forte pressione.

Il formaggio aveva così una consistenza ferma e secca e fu diviso in 15 parti che furono gettate sotto la pressa in apposite forme.

Il n. I esaminato immediatamente, ha dato la composizione del formaggio appena coagulato e pressato, e gli altri, che hanno ricevuto la salatura con sale in polvere uniformemente ripartito nella pasta prima di essere gittata in forma, furono esaminati susseguentemente alla distanza di un mese l'uno dall'altro. Al nono mese esaminando il formaggio n. 10 ho ottenuto risultati uguali a quelli avuti per il n. 9 esaminato nell'ottavo mese, il che mi indica che la maturazione si era completata all'ottavo mese appunto e la prima parte del lavoro è così esaurita.

Resta ora a vedere se questa maturazione che all'ottavo mese sembra completa, sia veramente tale o giunta a questo punto continua molto più lentamente, tanto che l'esame del formaggio non dà sensibili variazioni di composizione nel breve periodo di un mese. Inoltre, dato che il processo continui, esso ha un arresto definitivo o si collega con altro processo che trasforma diversamente i prodotti formati nella maturazione? Mi riservo di rispondere a ciò con altra nota poichè ho in corso esperienze in proposito.

Ho creduto opportuno esaminare, con le norme ordinarie, il latte che è servito alla preparazione del formaggio nonchè il prodotto secondario della preparazione stessa, il siero. Riporto quindi qui appresso i risultati ottenuti rispettivamente dall'uno e dall'altro esame.

COMPOSIZIONE CENTESIMALE DEL LATTE

Acqua	gr:	81.2817
Grasso	"	6.8107
Caseina	"	5.2716
Lattalbumina	"	1.0433
Lattosio	"	4.7106
Ceneri	"	0.8220
	"	99.9399

COMPOSIZIONE CENTESIMALE DEL SIERRO

Acqua	gr:	89.2449
Grasso	"	2.9703
Sost. proteiche	"	1.7645
Lattosio	"	4.1619
Ceneri	"	0.1670
Acidità (espressa in acido lattico)	"	0.0359
	"	98.9945

Esame Del Formaggio N. I.

(fresco = appena coagulato e pressato)

Caratteri Fisici = Massa bianca, secca e fragile, senza odore nè sapore sensibili. Messo su carta bibula non lascia traccia di corpo grasso.

La carta di tornasole indica reazione leggermente acida.

Determinazione Di Acqua = La determinazione dell'acqua fu fatta pesando esattamente una certa quantità di formaggio e seccandola prima nel vuoto su H_2SO_4 e poi in stufa a 110° fino a peso costante. Poichè il pecorino ha una pasta abbastanza dura, ho creduto conveniente operare la determinazione direttamente su di esso, senza aggiunta di sabbia, come generalmente si usa fare con altri formaggi.

Per quanto, sia nel vuoto su H_2SO_4, che in stufa a 110°, vadano via altre sostanze volatili, come NH_3, ed altri prodotti di decomposizione eventualmente presenti, pure la determinazione si può ritenere esatta, perchè questi sono in tale piccola quantità che si possono trascurare.

I risultati finali delle determinazioni sono riuniti nel seguente quadro:

Prove	H_2O %	
I	gr:	40.7518
II	"	41.4391
III	"	41.0574
Media	"	41.0828

Determinazione Delle Sostanze Grasse e Degli Acidi Grassi = Questa determinazione fu fatta estraendo con etere puro ed anidro il formaggio ridotto in piccoli pezzi e mantenuto in sacchetto di carta in apparecchio Soxlet.

Poichè l'etere estrae dal formaggio, insieme al grasso, l'acido lattico e gli acidi grassi eventualmente presenti, così l'estratto etereo, prima seccato e pesato, fu poi ripreso con etere, neutralizzando l'acidità con soluzione di carbonato sodico.—In tal modo i gliceridi restano nella soluzione eterea, mentre passano in quella acquosa, i saponi alcalini degli acidi grassi e l'acido lattico allo stato di lattato.

Separata la soluzione eterea, e ripetutamente lavata con acqua, fu determinato esattamente il contenuto in grasso neutro, distillando l'etere dalla soluzione eterea e pesando il residuo dopo averlo seccato a 110°.

Per differenza dall'estratto etereo si ha la somma degli acidi grassi liberi e dell'acido lattico (formati nella maturazione), dalla quale somma togliendo la quantità di acido lattico, che si determina a parte, si ha anche il contenuto in acidi grassi.

RISULTADO DELLE DETERMINAZIONI

Prove	Nel prodotto naturale			Grasso neutro per 100 nel prodotto seccato a 110°
	Estratto etereo %	Grasso neutro %	Ac. latt. acidi grassi %	
I	2.7391	1.8583	0.8808	3.1540
II	2.7902	1.8824	0.9078	3.1949
III	2.7454	1.8750	0.8704	3.1824
Media	2.7582	1.8719	0.8863	3.1771

La materia grassa, ottenuta per evaporazione dell'etere, aveva la più grande analogia col burro per il suo sapore e per la temperatura di fusione che era 28°—Credetti opportuno trattarla con soluzione alcoolica di KOH bollente per esaminare i prodotti

della sua saponificazione: ottenni, dopo saturazione dell'alcale con HCl diluito, delle laminette cristalline che, disseccate in carta bibula, abbandonarono a questa una certa quantità di sostanza oleosa, restando una materia che io potetti far cristallizzare sciogliendola in alcool. Le laminstte brillanti, micacee, che così ottenni, ricordavano le lamine dell'acido margarico. La sostanza oleosa, della quale s'era impregnata la carta non poteva essere altro che acido oleico; ma la quantità di materia sulla quale operavo, era troppo piccola per permettermi d'acquistare una nozione dompleta sulla natura della materia che presenta tutti i caratteri del burro. Era importante constatare che la quantità di grasso che si trova nel formaggio appena preparato non oltrepassa il 2 %, e che esso non può essere che del burro meccanicamente, trasportato mella preparazione.

DETERMINAZIONE DELL'ACIDITA RIFERITA IN ACIDO LATTICO = La determinazione fu fatta nel prodotto naturale adoperando circa gr: 10 di campione per ogni prova. 1 gr: 10 di sostanza venivano scaldati con acqua a più riprese, decantando ogni volta il liquido: i liquidi riuniti e filtrati furon portati a 200 cc., e sopra 100 cc. (circa gr: 5 di sostanza) fu titolata l'acidita con soluzione N—10 di KOH, indicatore il tornasole

I risultati sono riferiti nell'unito quadro:

Prove	Nel prodotto naturale			Acidita riferita ad acido lattico % nel prodotto seccato a 110°
	Estratto etereo %	Grasso neutro %	Ac. latt. ac. gras %	
I	0.7930	.....	.....	1.3459
II	0.9106	.....	.....	1.5455
Media	0.8518	0.8863	0.0345	1.4457

DETERMINAZIONE DEL LATTOSIO = Questa determinazione non ha grande importanza e non si esegue che nel formaggio freschissimo. Poichè è difficile estrarre completamente il lattosio con acqua leggermente scaldata, a circa gr: 50 di formaggio furono

aggiunti cc. 60 di una soluzione di NaOH diluitissima, leggermente scaldata, e in adatto recipiente fu continuato il mite riscaldamento per qualche ora.

Freddata la massa grassa fu forata e se ne separò la soluzione acquosa, ripetendo a più riprese il trattamento.

I liquidi acquosi separati si acidificano con acido citrico per precipitare la caseina e si filtrano. Il filtrato si porta a volume noto e su una parte aliquota di esso si procede direttamente al dosamento del lattosio col liquido di Fehling.

Riporto nel seguente quadro i risultati ottenuti:

Prove	Lattosio %	
	Nel prodotto naturale	Nel prodotto seccato a 110
I	2.1913	3.7192
II	2.2100	3.7510
III	2.1752	3.6919
Media	2.1921	3.7207

Determinazione Dell'Azoto Totale = L'azoto totale fu determinato col metodo Kyeldahl adoperando come ossidante, insieme all'H_2SO_4 conc., un miscuglio di p.1 di ossido di mercurio giallo, p.1 di solfato di rame e p.8 di solfato potassico. Distrutta la materia organica, furon precipitati allo stato di solfuro il mercurio ed il rame, prima della distillazione con ossido di magnesio, mediante apposita soluzione di solfuro sodico. Anche questa determinazione fu fatta sul prodotto naturale con i seguenti risultati:

Prove	Nel prodotto naturale		Nel prodotto seccato a 110°	
	N totale %	Sostanze azotate %	N totale %	Sostanze azotate %
I	8.5198	53.2487	14.2909	89.3181
II	8.4913	53.0706	14.4122	90.0762
III	8.5027	53.1418	14.4316	90.1975
Media	8.5046	53.1537	14.3782	89.8639

DETERMINAZIONE DELLE CENERI = La determinazione fu fatta sul prodotto naturale. Riporto i risultati nel seguente quadro:

Prove	Ceneri %	
	Nel prodotto natur.	Nel prodotto seccato a 110°
I	0.7618	1.2930
II	0.7392	1.2546
Media	0.7505	1.2738

Il pecorino dunque, appena coagulato e pressato ha la seguente composizione centesimale media:

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	41.0828	.....
Sostanze grasse	1.8719	3.1771
Acido lattico	0.8518	1.4457
Altri acidi grassi	0.0345	0.0585
Lattosio	2.1921	3.7207
Sostanze azotate	53.1537	89.8639
Ceneri	0.7505	1.2738
	99.9372	99.5397

Le varie determinazioni sui diversi formaggi, corrispondenti ai n. 2, 3, 4, 5, 6, 7, 8, 9 e 10 furon fatte (due o tre prove per ogni determinazione) seguendo i metodi precedentemente descritti per il n. 1, aggiungendo l'esame delle sostanze azotate, nonchè quello sulla natura del grasso formato dopo che il formaggio (n. 6) aveva acquistato tutti i caratteri esteriori che doveva comunicare ad esso la stagionatura—

Come si sa, le sostanze azotate del formaggio sono costituite da sostanze proteiche solubili ed insolubili, dai loro prodotti di decomposizione, da saponi e sali ammoniacali.

Questi diversi prodotti sono stati valutati nel modo seguente:

Ho pesato esattamente circa gr: 20 di formaggio, e, dopo averli pestati in un mortaio, vi ho aggiunto acqua a poco a poco, fino ad avere un volume uguale a circa il doppio di quello del campione—Ottenuta così una pasta omogenea, ho lasciato riposare per le ½ h. perchè si fossero imbevute bene di acqua tutte le particelle solide, indi ho aggiunto ancora dell'acqua agitando—Separata così la materia grassa, seguitai ad aggiungere acqua e versai il tutto in palloncino tarato da 250 cc. fino a raggiungere il volume = Agitai e lasciai riposare per 15 h. Chiarificatosi il liquido fu filtrato, raccogliendone cc. 200.

Su cc. 25 di questo liquido fu fatta la determinazione di azoto col solito metodo di Kyeldahl = Quest'azoto costituisce quello dell'estratto acquoso, cioè quello delle sostanze proteiche solubili e dei prodotti di decomposizione (basi amidiche e ammoniaca), che son pure solubili. Per differenza dall'azoto totale si ha quello delle sostanze proteiche insolubili.

Questi due dati hanno una grande importanza in quanto danno il peso ed il corrispondente valore in azoto della sostanza organica resa solubile dai processi fermentativi, ed il loro rapporto dà il *coefficiente di maturazione*—

Le sostanze proteiche sono state separate dai prodotti di decomposizione (basi ammidiche) impiegando l'acido fosfo-wolframico. La presenza di quest'acido non impedisce la determinazione di azoto col metodo Kyeldahl nel precipitato e nel liquido filtrato.

Cc. 50 del liquido precedentemente preparato furono portati a cc. 70–80 aggiungendo cc. 15 di HCl al 20 % e precipitando con fosfo-wolframato sodico. Raccolto sul filtro e lavato il precipitato con soluzione d'H_2SO_4 (5 %), poi con alcool assoluto, fu seccato, determinando l'azoto sul prodotto secco. Dalla quantità d'azoto trovata fu tolta quella corrispondente all'ammoniaca contenuta nella soluzione acquosa del formaggio, la quale è pure precipitata dall'acido fosfo-wolframico, e la differenza rappresentava quindi *l'azoto delle sostanze proteiche solubili nell'acqua*—

L'ammoniaca si determina a parte, su altri cc. 50 della soluzione primitiva, spostandola, all'ebollizione, con ossido di magnesio.

Il rapporto fra l'azoto delle sostanze proteiche dell'estratto acquoso a freddo e quello totale del formaggio rappresenta il *coefficiente di solubilizzazione*, cioè l'azione dei fermenti diastasici.—

La differenza fra l'azoto totale dell'estratto acquoso e quello delle sostanze proteiche precipitate con l'acido fosfo-wolframico costituisce *l'azoto dei prodotti di decomposizione.*

Il rapporto fra l'azoto non proteico dell'estratto acquoso e quello totale del formaggio rappresenta il *coefficiente di decomposizione*, cicè l'azione diretta dei microrganismi.

L'*azoto ammidico* è quello che risulta dall'azoto totale dell estratto acquoso detratto della somma dell'azoto delle sostanze proteiche solubili e dell'azoto ammoniacale.

Le basi ammidiche, provenienti dalla decomposizione della caseina, sono essenzialmente costituite di leucina e contengono solo in piccolissima quantità tirosina, butilammina, amilammina, ecc. fino ad arrivare all'ammoniaca. Per questo nel fare il calcolo delle basi ammidiche dall'azoto trovato, io ho potuto considerar questo come proveniente integralmente dalla leucina, senza tema di grave errore.

Sperimentalmente infatti ho potuto osservare il seguente fatto:

Il residuo del trattamento con etere per l'estrazione delle sostanze grasse e degli acidi grassi, ripreso con alcol a 36°, mi fornì, dopo evaporazione del solvente, dei cristalli madraperlacei di leucina, misti a piccolissime quantità di altri corpi colorati in giallo, che non ho esaminato sia per la scarsezza del materiale, sia perchè, allo scopo del lavoro, la loro conoscenza non importava gran che, potendosi dedurre approssimativamente la loro natura da quanto finora si sa sulla maturazione.

Ciò che resta del formaggio, dopo l'estrazione con etere e relativos eccamento è costituito da tutte le sostanze azotate organiche; ammoniaca, lattosio e sali minerali, naturali ed aggiunti con la salatura. Il residuo così costituito cede all'alcool a 36°, quando venga trattato con questo solvente, tutti i suoi costituenti ad eccezione della caseina: conoscendo quindi il % di azoto totale, nonchè quello ammoniacale, ed i % di lattosio e di sali minerali naturali ed aggiunti nel formaggio, ho determinato l'azoto totale dell'estratto alcoolco (36°), e sottraendo da esso l'azoto ammoniacale, determinato à parte, ho potuto conoscere

l'azoto ammidico, il quale corrisponde quasi esattamente a quello contenuto nella quantità trovata di leucina.

ESAME DEL FORMAGGIO N. 2

(fresco di un mese che ha subito la salatura)

Caratteri Fisici = Il formaggio che ha subito la salatura, coagulato e pressato da un mese, ha completamente cambiato d'aspetto. Esso ha già preso l'aspetto d'un corpo grasso e macchia la carta bibula sulla quale è deposto. Il suo sapore comincia ad esser dolce e piacevole, il suo odore appena sensibile.

Composizione Centesimale =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	37.5080	.....
Sostanze grasse	7.9349	12.7398
Acido lattico	0.8649	1.3887
Altri acidi grassi	0.7899	1.2683
Lattosio	2.1949	3.5177
Sostanze azotate	44.5937	71.5966
Ceneri e cloruro sodico	5.8099	9.3280
	99.6962	99.8391

Esme Sostanze Azotate =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	0.2529	1.5806	0.4060	2.5375
	Insolubili	6.5037	40.6481	10.4418	65.2612
Non proteiche sol	Ammoniacali.....	0.1436	0.1743	0.2305	0.3198
	Ammidiche Leucina	0.2348	2.970	0.3769	3.5266
Totali		7.1350	44.6000	11.4552	71.6451

I diversi coefficienti di solubilizzazione, decomposizione e maturazione, calcolati da questi dati, come è precedentemente detto, sono i seguenti:

COEFFICIENTE DI SOLUBILIZZAZIONE (dovuta all'azione delle disastasi)	0.0354
COEFFICIENTE DI DECOMPOSIZIONE (dovuta alla azione dei microrganismi)	0.0530
COEFFICIENTE DI MATURAZIONE	0.0970

Le sostanze azotate di questo formaggio hanno, secondo i dati analitici, la seguente composizione centesimale:

Caseina solubile	gr	3.5439
" insolubile	"	91.2289
Ammoniaca	"	0.3908
Basi ammidiche (leucina)	"	4.9260
	"	100.0896

Da questi risultati pare dimostrato che il soggiorno all'aria (per la maturazione) del pecorino ha per effetto di aumentare la quantità di materia grassa, che esso contiene in proporzione abbastanza considerevole; ma prima di esaminare la natura del grasso formato in queste condizioni, trovo conveniente attendere che il formaggio abbia acquistato tutte le qualità che un soggiorno più prolungato all'aria deve comunicargli.

La quantità di caseina invece, che prima era tutta insolubile, è considerevolmente diminuita, e di essa una parte, per azione delle diastasi, si è resa solubile, porzione della quale, per azione dei microrganismi, si è decomposta in basi ammidiche fino ad arrivare all'ammoniaca.

ESAME DEL FORMAGGIO N. 3

(fresco di due mesi che ha subito la salatura)

CARATTERI FISICI = Questo formaggio ha l'aspetto di un corpo grasso come il precedente: ma il suo sapore è più dolce e piacevole,

il suo odore più sensibile, proprietà che vanno sempre più accentuandosi nei formaggi corrispondenti ai successivi numeri 4, 5, 6, 7, 8, 9 e 10.

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	34.1019	.....
Sostanze grasse	13.9999	21.4644
Acido lattico	0.8799	1.3491
Altri acidi grassi	1.5399	2.3610
Lattosio	2.1949	3.3653
Sostanze azotate	40.3287	61.8284
Ceneri e cloruro sodico	6.2819	9.6314
	99.3271	99.9996

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	0.4888	3.0550	0.7494	4.6837
	Insolub.	5.2244	32.6525	8.0099	50.0618
Non prot. sol.	Ammoniacali	0.2799	0.3399	0.4291	0.5210
	Ammidiche (Leucina)	0.4595	4.2340	0.7044	6.5910
Totali		6.4526	40.2814	9.8928	61.8575

COEFFICIENTI =

di solubilizzazione	0.0757
di decomposizione	0.1145
di maturazione	0.2351

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	7.5841
" insolubile	"	81.0609
Ammoniaca	"	0.8438
Basi ammidiche (leucina)	"	10.5110
		99.9998

ESAME DEL FORMAGGIO N. 4

(salato di tre mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	34.0178	.....
Sostanze grasse	21.4021	32.6375
Acido lattico	1.9968	3.0451
Altri acidi grassi	1.9331	2.9480
Lattosio	1.1198	1.7077
Sostanze azotate	33.0625	50.1889
Ceneri e cloruro sodico	6.2115	9.4724
	99.7136	99.9996

ESAME SOSTANZE AZOTATE

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostazne azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	0.8823	5.5143	1.3393	8.3706
	Insolubili	3.4827	21.7668	5.2868	33.0425
Non prot. sol.	Ammoniacali	0.3530	0.4286	0.5358	0.6506
	Ammidiche (leucina)	0.5720	5.3522	0.8683	8.1246
Totali		5.2900	33.0619	8.0902	50.1883

COEFFICIENTI

di solubilizzazione	0.1667
di decomposizione	0.1748
di maturazione	0.3415

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	16.7089
" insolubile	"	65.8365
Ammoniaca	"	1.2963
Basi ammidiche (leucina)	"	16.1884
	"	100.0301

ESAME DEL FORMAGGIO N. 5

(salato di quattro mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9765	.....
Sostanze grasse	25.1742	38.1293
Acido lattico	2.5612	3.8793
Altri acidi grassi	2.1375	3.2375
Lattosio	0.5882	0.8909
Sostanze azotate	29.3750	44.3583
Ceneri e cloruro sodico	6.2750	9.5043
	100.1276	99.9996

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0180	6.3625	1.5372	9.6075
	Insolub.	2.6925	16.8281	4.0658	25.4112
Non prot. sol.	Ammoniacali	0.3897	0.4732	0.5884	0.7144
	Ammidiche (leucina)	0.5998	5.6187	0.9057	8.4746
Totali		4.7000	29.2825	7.0971	44.2077

COEFFICIENTI =

di solubilizzazione	0.2166
di decomposizione	0.2105
di maturazione	0.7455

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	21.7279
" insolubile	"	57.4681
Ammoniaca	"	1.6159
Basi ammidiche (leucina)	"	19.1879
		99.9998

ESAME DEL FORMAGGIO N. 6

(salato di cinque mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9551	.....
Sostanze grasse	27.2007	41.1852
Acido lattico	2.8578	4.3272
Altri acidi grassi	2.2502	3.4071
Lattosio	0.3238	0.4904
Sostanze azotate	27.0625	41.0895
Ceneri e cloruro sodico	6.2744	9.5003
	99.9245	99.9997

ESAME SOSTANZE AZOTATE

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0165	6.3531	1.5434	9.6462
	Insolub.	2.2456	14.0350	3.4096	21.3100
Non prot. sol.	Ammoniacali	0.4079	0.4953	0.6193	0.7520
	Ammidiche (leucina)	0.6600	6.1756	1.0021	9.3766
Totali		4.3300	27.0590	6.5744	41.0848

COEFFICIENTI =

di solubilizzazione	0.2347
di decomposizione	0.2466
di maturazione	0.9282

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	23.4786
" insolubile	"	51.8681
Ammoniaca	"	1.8304
Basi ammidiche (leucina)	"	22.8227
		99.9998

ESAME DEL FORMAGGIO N. 7

(salato di sei mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9884	.....
Sostanze grasse	28.1554	42.6265
Acido lattico	2.9998	4.5417
Altri acidi grassi	2.3025	3.4860
Lattosio	0.1906	0.2886
Sostanze azotate	25.9875	39.4741
Ceneri e cloruro sodico	6.3289	9.5818
	99.9131	99.9987

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 100° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0234	6.3962	1.5545	9.7156
	Insolubili	2.0395	12.7468	3.0979	19.2223
Non prot. sol.	Ammoniaca	0.4172	0.5066	0.6337	0.7695
	Ammidiche (leucina)	0.6779	6.3431	1.0297	9.6349
Totali		4.1580	25.9927	6.3158	39.3423

COEFFICIENTI =

di solubilizzazione	0.2461
di decomposizione	0.2633
di maturazione	1.0386

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	24.6076
" insolubile	"	49.0339
Ammoniaca	"	1.9490
Basi ammidiche (leucina)	"	24.4033
		99.9938

ESAME DEL FORMAGGIO N. 8

(salato di sette mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9432	.....
Sostanze grasse	28.5344	43.1968
Acido lattico	3.0729	4.6520
Altri acidi grassi	2.3287	3.5253
Lattosio	0.1245	0.1885
Sostanze azotate	25.8062	39.2319
Ceneri e cloruro sodico	6.2814	9.5092
	100.0913	100.3037

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina	Solubili	1.0294	6.4337	1.5649	10.0797
	Insolub	1.9948	12.4675	3.0376	18.9537
Non prot. sol.	Ammoniacali	0.4216	0.5119	0.6409	0.7782
	Ammidiche (leucina)	0.6832	6.3927	1.0386	9.7181
Totali		4.1290	25.8058	6.2780	39.5297

COEFFICIENTI =

di solubilizzazione	0.2493
di decomposizione	0.2675
di maturazione	1.0698

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	24.9312
" insolubile	"	48.3127
Ammoniaca	"	1.9836
Basi ammidiche (leucina)	"	24.7723
		99.9998

ESAME DEL FORMAGGIO N. 9

(salato di otto mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9415	.....
Sostanze grasse	29.1974	44.0852
Acido lattico	3.1319	4.7579
Altri acidi grassi	2.3399	3.5547
Lattosio	0.0529	0.0805
Sostanze azotate	25.0000	37.9783
Ceneri e cloruro sodico	6.2820	9.5433
	99.9456	99.9999

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0297	6.4356	1.5642	9.7762
	Insolub.	1.8586	11.6162	2.8234	17.6462
Non prot. sol.	Ammoniacali	0.4249	0.5159	0.6439	0.7818
	Ammidiche (leucina)	0.6868	6.4263	1.0433	9.7621
Totali		4.0000	24.9940	6.0748	37.9663

COEFFICIENTI =

di solubilizzazione	0.2574
di decomposizione	0.2779
di maturazione	1.1521

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	25.7485
" insolubile	"	46.4759
Ammoniaca	"	2.0640
Basi ammidiche (leucina)	"	25.7113
		99.9997

ESAME DEL FORMAGGIO N. 10

(salato di nove mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9421	.....
Sostanze grasse	29.1810	44.1749
Acido lattico	3.1504	4.7693
Altri acidi grassi	2.3595	3.5720
Lattosio	0.0573	0.0868
Sostanze azotate	25.0000	38.0818
Ceneri e cloruro sodico	6.1531	9.3148
	99.8434	99.9996

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0346	6.4662	1.5759	9.8493
	Insolub.	1.8401	11.5006	2.8029	17.5181
Non prot. sol.	Ammoniacali	0.4264	0.5177	0.6495	0.7885
	Ammidiche (leucina)	0.6989	6.5396	1.0646	9.9614
Totali		4.0000	25.0929	6.0929	38.1174

COEFFICIENTI =

di solubilizzazione	0.2586
di decomposizione	0.2813
di maturazione	1.1737

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	25.8398
" insolubile	"	45.9580
Ammoniaca	"	2.0688
Basi ammidiche (leucina)	"	26.1332
		99.9998

Riassumo nei seguenti quadri i risultati analitici di tutti i formaggi esaminati sostituendo alle sostanze azotate totali, calcolate dall'azoto trovato, quelle risultanti dall'esame di esse.

COMPOSIZIONE CENTESIMALE DEL PRODOTTO NATURALE=

Numeri corrispondenti ai campioni	1	2	3	4	5	6	7	8	9	10
Acqua	41.0828	37.5080	34.1019	34.0178	33.9765	33.9551	33.9484	33.9432	33.9415	33.9421
Sostanze grasse neutre	1.8719	7.9349	13.9999	21.4021	25.1742	27.2007	28.1554	28.5344	29.1974	29.1810
Acido lattico	0.8518	0.8649	0.8799	1.9968	2.5612	2.8578	2.9998	3.0729	3.1319	3.1504
Altri acidi grassi	0.0345	0.7899	1.5399	1.9331	2.1375	2.2502	2.3025	2.3287	2.3399	2.3595
Lattosio	2.1921	2.1949	2.1949	1.1198	0.5882	0.3238	0.1906	0.1245	0.0529	0.0573
Sost. azotate — Caseina solubile	.....	1.5806	3.0550	5.5143	6.3625	6.3531	6.3962	6.4337	6.4356	6.4662
Sost. azotate — " insolubile	.....	40.6481	32.6525	21.7668	16.8281	14.0350	12.7468	12.4675	11.6162	11.5006
Sost. azotate — Ammoniaca	53.1537	0.1743	0.3399	0.4286	0.4732	0.4953	0.5066	0.5119	0.5159	0.5177
Sost. azotate — Basi ammidiche (leucina)	.....	2.1970	4.2340	5.3522	5.6187	6.1756	6.3431	6.3927	6.4263	6.5396
Sost. minerali (Ceneri e NaCl)	0.7505	5.8099	6.2819	6.2115	6.2750	6.2744	6.3289	6.2814	6.2820	6.1531
	99.9383	99.7025	99.2798	99.7430	99.9951	99.9210	99.9183	100.0909	99.9396	99.8675

COEFFICIENTI

Di solubilizzazione	.....	0.0354	0.05757	0.4667	0.2166	0.2347	0.2461	0.2493	0.2574	0.2585
Di decomposizione	.....	0.0530	0.1145	0.1748	0.2105	0.2466	0.2633	0.2675	0.2779	0.2813
Di maturazione	.....	0.0970	0.2351	0.3415	0.7455	0.9282	1.0386	1.0698	1.1521	1.1737

COMPOSIZIONE CENTESIMALE DEL SECCATOA A 110°=

N. corrispondenti ai campioni	1	2	3	4	5	6	7	8	9	10
Acqua	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Sostanze grasse neutre	3.1771	12.7398	21.4644	32.6375	38.1293	41.1852	42.6265	43.1968	44.0852	44.1749
Acido lattico	1.4457	1.3887	1.3491	3.0451	3.8793	4.3272	4.5417	4.6520	4.7579	4.7693
Altri acidi grassi	0.0585	1.2683	2.3610	2.9480	3.2375	3.4071	3.4860	3.5253	3.5547	3.5720
Lattosio	3.7207	3.5177	3.3653	1.7077	0.8909	0.4904	0.2086	0.1885	0.0805	0.0868
Sost. azotate — Caseina solubile	.....	2.5375	4.6837	8.3706	9.6075	9.6462	9.7156	10.0797	9.7762	9.8493
Sost. azotate — " insolubile	.....	65.2612	50.0618	33.0425	25.4112	21.3100	19.2233	18.9537	17.6462	17.5181
Sost. azotate — Ammoniaca	89.8639	0.3198	0.5210	0.6506	0.7144	0.7520	0.7695	0.7782	0.7818	0.7886
Sost. azotate — Basi ammidiche (leuc.)	.....	3.5266	6.5910	8.1246	8.4746	9.3766	9.6349	9.7181	9.7621	9.9614
Sost. minerali (Ceneri e NaCl)	1.2738	9.3280	9.6314	9.4724	9.5043	9.5003	9.5818	9.5092	9.5433	9.3148
	99.5397	99.8876	100.0287	99.9990	99.8490	99.9950	99.8669	100.6015	99.9879	100.0352

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE=

Caseina solubile	.....	3.5439	7.5841	16.7089	21.7279	23.4786	24.6076	24.9312	25.7485	25.8398
' insolubile	.....	91.2289	81.0609	65.8365	57.4681	51.8681	49.0339	48.3127	46.4759	45.9580
Ammoniaca	.....	0.3908	0.8438	1.2963	1.6159	1.8304	1.9490	1.9836	2.0640	2.0688
Basi ammidiche (leucina)	.....	4.9260	10.5110	16.1884	19.1879	22.8227	24.4033	24.7723	25.7113	26.1332
	.....	100.0896	99.9998	100.0301	99.9998	99.9998	99.9938	99.9998	99.9997	99.9998

Dal confronto delle precedenti analisi si vede chiaramente che nella maturazione la sostanza grassa aumenta, rapidamente nei primi mesi e molto più lentamente in seguito con una percentuale che dal 2 % circa nel formaggio fresco si eleva fino ad oltre il 29 % nel prodotto maturo. In relazione a quest'aumento è la diminuzione, per quanto non proporzionale, delle sostanze azotate, le quali, diminuendo, mutano anche natura, poichè si solubilizzano e si decompongono fino ad arrivare all'ammoniaca, prodotto che viene in parte fissato dagli acidi grassi provenienti dall'ossidazione dell'oleina, costituente, come dimostrerò in seguito, della sostanza grassa contenuta nel formaggio.

In diminuzione, per la stagionatura, è pure il lattosio; ma proporzionalmente in aumento è l'acido lattico, il che mostra la trasformazione del primo nel secondo prodotto.

Osservando poi la composizione centesimale delle sostanze azotate all'inizio ed a fine maturazione si vede come queste, essenzialmente costituite in principio di caseina insolubile, vadano gradualmente trasformandosi, tanto che a fine maturazione la caseina insolubile non costituisce che il 46 % circa delle sostanze azotate totali, mentre il resto risulta trasformato parte in caseina solubile, parte in ammoniaca e basi ammidiche (leucina) con preponderanza di queste ultime.

I coefficienti di solubilizzazione e di decomposizione, in relazione con le trasformazione che man mano subiscono le sostanze azotate, crescono entrambi, ma non proporzionatamente l'uno all'altro, e mentre all'inizio della maturazione il primo ha un valore numerico di poco superiore alla metà del secondo, alla fine diventano quasi uguali mantenendosi sempre però una lievissima prevalenza del secondo sul primo; La loro somma inoltre, che a principio rappresenta quasi il coefficiente di maturazione, nel prodotto stagionato è inferiore alla metà del valore numerico di quest'ultimo coefficiente—

Da quest'osservazione si può trarre un'utilissima conclusione circa il giudizio sul grado di maturazione di un formaggio, e cioè UN PRODOTTO E'TANTO PIU'MATURO QUANTO PIU'VICINI FRA LORO SONO I VALORI DEI COEFFICIENTI DI SOLUBILIZZAZIONE E DI DECOMPOSIZIONE (il primo deve sempre essere inferio-

re al secondo) E QUANTO PIU'LA LORO SOMMA E'INFERIORE ALLA META'DEL COEFFICIENTE DI MATURAZIONE.

Dietro i suddetti risultati dunque non si può avere più dubbio: della materia grassa si è formata a spesa della caseina, durante la maturazione.

Prima di discutere il modo come, io credo, questa materia abbia potuto formarsi ho cercato di precisarne bene la natura.

Il formaggio n. 6 (salato di cinque mesi) possedeva tutte le qualità che esso era suscettibile di acquistare. Untuoso al gusto, presentava, tagliato col coltello, un aspetto grasso. Esso non si divideva più in frammenti e macchiava la carta sulla quale si deponeva. Possedeva inoltre un debole odore non notato nei formaggi precedenti, e credetti quindi opportuno fare l'esame del grasso in esso contenuto.

Per preparare la materia grassa da servire per l'esame chimico ho adottato il metodo del Laboratorio Chimico di Washington, che qui appresso descrivo:

Gr. 300 di formaggio, ridotti in frammenti della grandezza di un pisello, si trattano con 700 cc. di potassa (50 %) a 20° in una bottiglia a bocca larga, promuovendo la dissoluzione della caseina con una forte agitazione.

In dieci minuti la caseina è sciolta, ed il grasso viene alla superficie in piccole masse. Scuotendo il recipiente si fa in modo che il grasso si raduni in una massa sola, e con l'aggiunta, nel recipient di acqua fresca, il grasso raggiunge il collo del vaso, dal quale si può togliere con un cucchiaio. In tale operazione esso non viene attaccato, e, lavato con molta acqua per asportare tutto il residuo non grasso che vi si può trovare, in poco tempo è completamente separato, e si può preparare, filtrandolo ed asciugandolo, come è prescritto per l'esame delle materie grasse—

Questo grasso, che si trovava presente nel formaggio, nella proporzione del 27, 2007 %, sciolto in alcool bollente, ha abbandonato, per raffreddamento, dei piccoli cristalli di una sostanza bianca, che, purificata per diversi trattamenti con alcool, ha fornito dei cristalli di aspetto madraperlaceo, costituiti di margarina pura: fondono infatti a 41°, e sottoposti all'azione di una temperatura elevata si decompongono dando luogo ad acroleina.

Per la sua caratterizzazione ho eseguito sulla sostanza le determinazioni seguenti coi risultatti che qui sotto trascrivo:

Indice di saponificazione	198.0
Indice di acidità	0.0
Numero degli eteri	198.0
" di jodio	0.0
" degli acidi volatili	0.0
" degli acidi fissi	95.51

Questi caratteri sono abbastanza precisi per provare che la sostanza grassa, formata a spese della caseina, contiene della margarina; ma per maggiore sicurezza, ed a conferma di ciò, ho sottoposto ad analisi la sostanza che ero riuscito ad isolare, nonchè l'acido grasso proveniente dalla sua saponificazione.

ANALISI DELLA SOSTANZA GRASSA FUSIBILE A 41°

I. Gr. O. 2962 di sostanza danno gr. O. 8284 di CO_2 e gr. O. 3269 di H_2O.

II. Gr. O. 3062 di sostanza danno gr. O. 8562 di CO_2 e gr. O. 3387 di H_2O.

E calcolando il %:

Trovato		Calcolato per $C_{51}H_{104}O_6$
I	II	
C=76.26	76.25	76.41
H=12.26	12.28	12.26
O= ...	.....	11.33
		100.00

Per ottenere l'acido grasso corrispondente ho saponificato con KOH ed ho poi decomposta l'emulsione saponosa con H_2SO_4 diluito. L'acido grasso, così messo in libertà, si riunisce alla superficie del liquido e comincia ad ammassarsi verso 40°.— Questa sostanza, sciolta in alcool bollente, dà, per raffreddamento, delle pagliette madraperlacee, che fondono a 58°—60°.

ANALISI DELL'ACIDO FUSIBILE A 59°

I. Gr. 0.3292 di sostanza danno gr. 0.9100 di CO_2 e gr. 0.3736 di H_2O.

II. Gr. 0.1905 di sostanza danno gr. 0.5270 di CO_2 e gr. 0.2159 di H_2O.

Calcolando il %:

Trovato		Calcolato per $C_{17}H_{34}O_2$
I	II	
C=75.38	75.43	75.55
H=12.60	12.58	12.59
O= ...	.....	11.86
		100.00

La sostanza grassa contenuta nel pecorino, dietro questi risultati, non è che margarina, accompagnata da un altro corpo grasso, maggiormente solubile in alcool, che si ritrova nel solvente dopo che si è deposta, per raffreddamento, la margarina.

L'alcool evaporato a h.m. ha lasciato, come residuo, un olio leggermente giallastro, di sapore dolce e untuoso, liquido alla temperatura ordinaria. Quest'olio, scaldato, si decompone a 260° dando luogo a vapori di acroleina.

Su di esso ho eseguito, come sul precedente grasso solido, le seguenti determinazioni coi risultati che trascrivo:

Indice di saponificazione	190.0
" di acidità	0.0
Numero degli eteri	190.0
" di jodio	83.93
" degli acidi volatili	169.68
" " " fissi	0.0

Sottoposto ad analisi tha dato i seguenti risultati:

I. Gr. 0.2341 di sostanza danno gr. 0.6621 di CO_2 e gr. 0.2453 di H_2O.

II. Gr. 0.2785 di sostanza danno gr. 0.7901 di CO_2 e gr. 0.2914 di H_2O.

III. Gr. 0.1975 di sostanza danno gr. 0.5602 di CO_2 e gr. 0.2074 di H_2O.

Calcolando il %:

Trovato			Calcolato per $C_{57}H_{104}O_6$
I	II	III	
C=77.12	77.36	77.35	77.37
H=11.64	11.62	11.66	11.76
O= ...	.....	.....	10.87
			100.00

Quantunque la proprietà e l'analisi dimostrino che la sostanza liquida che accompagna la margarina non è che oleina, ho voluto saponificarla, e fare l'analisi dell'acido grasso proveniente da questa saponificazione.

L'olio ottenuto dall'evaporazione dell'alcool, dal quale si era separata la margarina, è stato saponificato con soda e l'emulsione decomposta con H_2SO_4 diluito—Si è così ottenuta una sostanza bianca leggermente giallastra, di sapore dolce e untuoso, che resta liquida fino a—10°.

Sottoposta ad analisi ha dato i seguenti risultati:

I. Gr. 0.2202 di sostanza danno gr. 0.6170 di CO_2 e gr. 0.2405 di H_2O—

II. Gr. 0.2005 di sostanza danno gr. 0.5620 di CO_2 e gr. 0.2182 di H_2O.

Calcolando il %:

Trovato		Calcolato per $C_{18}H_{34}O_2$
I	II	
C=76.41	76.43	76.59
H=12.11	12.08	12.05
O= ...	.....	11.36
		100.00

Questi risultati dimostrano che la sostanza grassa contenuta nel pecorino è un miscuglio di margarina ed oleina con prevalenza della prima, giacchè dei gr. 27, 2007 di grasso contenuto in gr. 100 di formaggio, gr. 15, 4337 sono di margarina e gr—11,7570 di oleina.

Dalle analisi precedenti si deduce che la caseina, nella maturazione, si è in parte trasformata in materia grassa, e ciò che è evidente è la singolare coincidenza che la sostanza grassa del burro è formata egualmente di margarina ed oleina presso a poco nelle stesse proporzioni nelle quali queste due sostanze costituiscono il grasso del formaggio, il che fa pensare che la materia grassa del burro si sia formata, nell'economia, a spese della caseina per reazioni analoghe a quelle che nel formaggio han dato luogo alla sostanza grassa che ivi si trova.

I formaggi n. 7, 8, 9 e 10, specie questi ultimi—(9 e 10), conservati oltre il quinto mese, mostrano nei loro caratteri esteriori delle profonde modificazioni. Il colore non tarda molto ad alterarsi, passa dal bianco al bruno, ed il formaggio prende un odore sempre più forte. Cambia anche il suo sapore e finisce per acquistare un gusto forte e piccante.

Importava conoscere le cause di queste modificazioni, ed ho voluto quindi esaminare anche il grasso del n.10, come avevo fatto per il n.6, poichè avevo notato che dopo il quinto mese la produzione di esso non aumentava più sensibilmente, come nei primi cinque mesi, pur rendendosi la percentuale delle sostanze azotate sempre più piccola.

La sostanza grassa nel formaggio n.10 è presente nella proporzione del 29 %. Di essa ho preparato un campione con lo stesso metodo avanti descritto, e l'ho sottoposto allo stesso trattamento. 1 gr. 29 di sostanza grassa, di color giallo-carico, li ho trovati costituiti di gr. 26.657 di margarina e di gr: 2,343 di oleina.

Questi risultati dimostrano che una parte del grasso che entra nella costituzione del pecorino si è decomposta, mentre si è formato del grasso nouvo, ed è sopratutto l'oleina che ha subito una profonda modificazione a contatto dell'aria.

Bisognava quindi andare alla ricerca, separazione e dosamento

di questi prodotti di ossidazione dell'oleina e per far ciò ho operato nel modo seguente:

Ho scaldato gr. 500 di formaggio, ridotto a piccoli pezzi, con circa 5 litri di acqua, a più riprese, decantando ogni volta il liquido = i liquidi riuniti e concentrati a un litro furon fatti freddara e filtrati. Il filtrato aveva preso una tinta giallastra e possedeva inoltre un odore forte ed un sapore piccante che ricordava quello del formaggio. Ho aggiunto al liquido acqua di barite ed ho poi distillato: si è formato un abbondante precipitato con sviluppo contemporaneo di odore ammoniacale. I vapori sviluppati condensati erano alcalini e contenevano dell'ammoniaca, della quale ho constatato la reazione alcalina e l'effetto su una bacchetta di vetro gabnata d'HCl.

L'ammoniaca era stata già determinata nell'esame delle sostanze azotate e non mi son quindi curato di determinarla ancora quì: essa fu trovata in quantità sufficiente per saturare tutti gli acidi grassi presenti, pure a parte complessivamente determinati.

Ho evaporato dunque il liquido a piccolo volume, e durante l'evaporazione si son depositati parte dei sali di bario che ho cercato di separare gli uni dagli altri impiegando il metodo Lerch, profittando cioè della loro differente solubilità in acqua calda—

Quando il liquido fu ridotto ad 1/10 circa del volume primitivo l'ho filtrato per separare i sali di bario depositati durante l'evaporazione, e nel filtrato si son prodotti, per il raffreddamento, degli aghetti riuniti in fasci assai voluminosi del peso di gr: 5,9192; i quali sottomessi ad analisi han dato i seguenti risultati:

I. Grm: 0.4834 di sostanza danno gr. 0.6931 di CO_2 e grm. 0.2663 di H_2O
II. Grm: 0.4396 di sostanza danno gr. 0.6309 di CO_2 e grm. 0.2430 di H_2O
III. Grm: 0.9106 di sostanza fanno gr. 0.5773 di $BaSO_4$ corrispondente a gr. 0.33939 di Ba
IV. Gr: 0.8996 di sostanza danno gr: 0.5708 di $BaSO_4$ corrispondente a gr. 0.33557 di Ba

Calcolando il %:

Trovato				Calcolato per Ba $(C_6H_{11}O_2)_2$
I	II	III	IV	
C =39.09	39.13	.....	....	39.23
H = 6.12	6.14	.....	.....	5.99
O =.....	.....	.....	.....	17.46
Ba=.....	.....	37.27	37.30	37.32
				100.00

Dai risultati di quest'analisi la sostanza solubile nell'acqua bollente ridotta a piccolo volume era il sale di bario dell'acido caproico, acido trovato nei prodotti d'ossidazione dell'acido oleico.

Le acque madri non più suscettibili di cristallizzazione le ho trattate con H_2SO_4 ed ho ottenuto, per distillazione, un acido incoloro che ricorda, per l'odore, il burro rancido, per cui ho pensato che potesse essere dell'ossido butirrico, opinione che mi è stata confermata dall'analisi del suo sale di argento che ho ottenuto precipitando una soluzione alcolica di $AgNO_3$ col liquido acido raccolto nella distillazione.

Il sale d'argento così ottenuto, lavato e seccato, pesava gr. 7.4432, il che corrisponde a gr. 3,3590 d'acido butirrico, acido che entra nella composizione di questo sale così come lo dimostra la seguente analisi.

I. Gr. 0.6292 di sostanza danno gr. 0.5665 di CO_2, gr. 0.2056 di H_2O e gr. 0.3480 di Ag.

II. Gr. 0.4851 di sostanza danno gr. 0.4365 di CO_2, gr. 0.1599 di H_2O e gr. 0.2684 di Ag.

Calcolando il %:

Trovato		Calcolato per $AgC_4H_7O_2$
I	II	
C =24.55	24.53	24.61
H = 3.63	3.66	3.58
O =.....	.....	16.43
Ag=55.30	55.32	55.38
		100.00

Per questi dati sperimentali posso concludere che l'acido butirrico è nel numero dei prodotti contenuti nel liquido filtrato.

I prodotti rimasti nel filtro l'ho addizionati con una certa quantità di H_2O e li ho messi a bollire, filtrando poi il liquido bollente. La mescolanza sulla quale operavo venne così divisa in due parti = una solubile in H_2O bollente, dalla quale la separai per evaporazione a b.m., l'altra insolubile. Quest'ultima parte, seccata e pesata, era gr. 3.2672 corrispondenti a gr. 2.3530 di acido caprico, poichè il sale di bario sul quale operavo era del caprato di bario come dimostra l'analisi:

I. Gr. 0.3564 di sostanza danno gr. 0.6527 di CO_2 e gr. 0.2564 di H_2O.

II. Gr. 0.3118 di sostanza danno gr. 0.5712 di CO_2 e gr. 0.2246 di H_2O.

III. Gr. 0.7115 di sostanza danno gr. 0.3456 di $BaSO_4$ corrispondenti a gr. 0.203178 di Ba.

IV. Gr. 0.7985 di sostanza danno gr. 0.3889 di $BaSO_4$ corrispondenti a gr. 0.2286 di Ba.

Calcolando il %:

Trovato				Calcolato per $Ba(C_{10}H_{19}O_2)_2$
I	II	III	IV	
C = 49.94	49.95	.....	.....	50.10
H = 7.99	8.00	.....	.....	7.93
O = ...	.....	.....	.....	13.37
Ba = ...	.....	28.55	28.63	28.60
				100.00

Anche dell'acido caprico è stata notata da Redtenbacher la presenza nei prodotti d'ossidazione, per NHO_3, dell'acido oleico:

La parte solubile in H_2O bollente fu ottenuta per evaporazione del liquido: il suo peso era di gr: 3.2047.

Sottomessa ad analisi ha condotto ai seguenti risultati:

I. Gr. 0.3386 di sostanza danno gr. 0.5618 di CO_2 e gr. 0.2186 di H_2O.

II. Gr. 0.3058 di sostanza danno gr. 0.5079 di CO_2 e gr. 0.1970 di H_2O.

III. Gr. 0.7984 di sostanza danno gr. 0.4390 di $BaSO_4$ corrispondenti a gr. 0.25808 di Ba.

IV. Gr. 0.8125 di sostanza danno gr. 0.4472 di $BaSO_4$ corrispondenti a gr. 0.2629 di Ba.

Calcolando il %:

Trovato				Calcolato per Ba $(C_8H_{15}O_2)_2$
I	II	III	IV	
C =45.24	45.29	.....	.....	45.39
H = 7.17	7.15	.....	.....	7.09
O =.....	.....	.....	.....	15.14
Ba=.....	.....	32.32	32.35	32.38
				100.00

I risultati di quest'analisi mi condussero ad ammettere che la sostanza analizzata era il sale di bario dell'acido caprilico anch'esso trovato nei prodotti d'ossidazione dell'acido oleico.

Riassumendo, i sali di bario che son riuscito a separare gli uni dagli altri profittando della loro differente solubilità in H_2O bollente, sono: Butirrato, Caproato, Caprato e Caprilato di Bario. Ho inoltre pesato questi differenti sali, i quali sono presenti nelle seguenti proporzioni in gr. 500 di formaggio:

Butirrato d'argento gr. 7.4432 corrispondenti a gr. 3.3590 di ac. butirrico

Caproato di bario gr. 5.9192 corrispondenti a gr. 3.7515 di ac. caproico

Caprato di bario gr. 3.2672 corrispondenti a gr. 2.3530 di ac. caproico

Caprilato di bario gr. 3.2047 corrispondenti a gr. 2.1820 di ac: caprilico.

Calcolando il % in acidi liberi si ha:

Acido butirrico	gr.	0.6718
" caproico	"	0.7503
" caprico	"	0.4706
" caprilico	"	0.4364
	"	2.3291

Dopo ciò, poichè nel formaggio (maturo) questi acidi sono saturati dall'ammoniaca, si può rappresentare la composizione del formaggio n. 10 nella seguente maniera:

Acqua	gr.	33.9421
Margarina	"	26.6570
Oleina	"	2.3430
Butirrato d'ammonio	"	0.8015
Caproato "	"	0.8602
Caprilato "	"	0.4879
Caprato "	"	0.5752
Acido lattico	"	3.1504
Lattosio	"	0.0573
Caseina solubile	"	6.4662
" insolubile	"	11.5006
Basi ammidiche (leucina)	"	6.5396
Sostanze minerali (ceneri e Nacl)	"	6.1581
		99.5391

Sono dunque autorizzato a dire che nel pecorino conservato per nove mesi a contatto dell'aria si trovano, indipendentemente dalla margarina e dalla oleina, tutti i prodotti d'ossidazione di quest'ultima sostanza, e poichè l'oleina che si trova nel formaggio di cinque mesi è in buona parte sparita, bisogna concludere che gli acidi butirrico, caprico, caprilico e caproico si originano dall'ossidazione di questa sostanza.

Tutti questi acidi si trovano ugualmente nel burro invecchiato con la differenza che nel formaggio essi sono saturati dall'ammoniaca, e sono appunto questi sali ammoniacali che danno al formaggio un sapore differente da quello del burro rancido, nel quale

gli acidi sono gli stessi, ma allo stato libero, non saturati da alcuna base.

Nell'epoca in cui Chevreul intraprese il suo celebre lavoro sui corpi grassi, egli si occupò dello studio del burro e trovò in questa sostanza, divenuta rancida, gli acidi butirrico, caprico e caproico. Malgrado lo stato imperfetto nel quale si trovava in quell'epoca l'analisi organica egli seppe perfettamente distinguere questi acidi e ne fece anche uno studio abbastanza completo. Dopo d'allora un gran numero di scienziati si è occupato dello stesso argomento e ricordo specialmente Lerch, il quale dopo aver saponificato il burro rancido lo distillò con un eccesso d'H_2SO_4 diluito, guingendo così ad ottenere fino a cinque acidi volatili (butirrico, caproico, caprico, caprilico e vaccinico).

M. Bromeis[1] ha studiato egualmente la costituzione del burro e vi ha trovato, indipendentemente dalla margarina e dall'oleina, dell'acido butirrico.

Dall'accordo di questi risultati con quelli che io stesso ho ottenuto si vede chiaramente che bisogna attribuire all'oleina l'irrancidimento del burro, come indubbiamente è all'ossidazione di questa sostanza che bisogna attribuire la produzione dei differenti acidi dei quali ho potuto constatare la presenza nel pecorino. La sola differenza che sembra esistere è che nel burro questi acidi sono allo stato libero, mentre nel formaggio essi sono combinati all'ammoniaca.

Credo così d'aver dimostrato che la caseina si trasforma, nella maturazione del formaggio, in una sostanza grassa avente la più grande analogia col burro, poichè si compone di margarina ed oleina, e queste sostanze entrano nella sua costituzione presso a poco nelle stesse proporzioni nelle quali esse si trovano nel burro.

Resta ora a stabilire come questa trasformazione avviene, ciò che mi propongo di fare in una prossima nota.

Napoli, Istituto di Chimica Generale della R. Università— Maggio del 1912.

[1] Ann. der Chem. und Pharm. XLII, 46.

THE GRINDING OF CORN-MEAL FOR BREAD

By F. P. Dunnington
University of Virginia, Charlottesville, Va.

At the present time, when the high prices of food occasion so much concern, and a conservation of all the resources of this country awakens so much interest, it is somewhat amazing that the United States produces such an enormous crop of Indian Corn and yet in the larger portion of this Country it is consumed in the form of bread to a very small extent.

Dr. Charles D. Woods, Director of the Maine Agricultural Experiment Station, has compiled an excellent treatise on the Food Value of Corn and Corn Products, published as Farmers' Bulletin No. 298 by the U. S. Department of Agriculture 1907; and in this he sets forth most plainly the advantages of composition, digestibility, wholesomeness, convenience and pecuniary economy of corn as a food for man.

The Encyclopedia Britannica 11th Ed. p. 449 states: "As an article of food maize is one of the most extensively used grains of the world. Although rich in nitrogenous matter and fat it does not make good bread."

It is generally understood, and so far as I have been able to obtain reports, it appears that in the U. S. the considerable use of corn as a bread is confined to the southern States and there largely to the population of the country, and smaller towns. In many of these localities it is more largely used than is wheat.

The readiness with which it may be prepared and the rapid and simple methods by which it may be cooked, as well as the pleasant and satisfying character of the food, its composition approaching to that of a complete ration, have much effect in determining its uses as a staple food.

On the other hand, a considerable amount of corn meal is made throughout the Central and Western States, especially at the

larger business centres—but it is used only sparingly;—often in admixture with wheat flour and largely in the form of mush (or hasty pudding). A few estimate its use as one fiftieth, and more at one hundredth of that of wheat flour.

In 1890 the U. S. Secretary of Agriculture, Hon. J. M. Rusk, endeavored to induce a larger use of corn a as bread stuff in Europe and made some expenditures under the efficient management of Mr. Chas. J. Murphy as a Special Agent, but does not seem to have succeeded, and there is probably even a smaller proportional consumption now in the United States than there was at that date in the form of bread, while the manufacture of grits and other corn products has been largely increased.

It is my endeavor in this study to ascertain, why this apparent inconsistency; that this cheaper, healthy food is so sparingly consumed, where economy in living is of so great import.

The question seems narrowed down to the manner of making the meal as being the factor which determines the extent of its use; and hence this, the narrow range of the discussion in this paper.

As to the grain itself, the comparatively high fat content, viz., 5 per cent. constitutes a considerable portion of its food value, while its presence adds to the care required in curing and keeping the grain, and also to the difficulty of keeping the meal for more than three or four weeks in most climates.

Hence it is that those supplying distant trade and most of the larger mills, find it best to kiln-dry the grain, so destroying any bacteria which may have infected it, and in grinding it, to remove the germ, so as to obtain a product freed in large measure from these drawbacks, thus treating both yellow and white corn.

The grinding is conducted by water, steam, or electricity for power, and we might judge their use as entirely a matter of indifference, yet that is not wholly so, as I shall have occasion to mention later on.

The mills employed formerly were only burr-stones (or occasionally made of some local sandstone), but in recent years, most of the larger mills use steel rollers, similar to those used for wheat flour.

Samples of meal have been secured from most of the corn pro-

ducing states, and to avoid advertising or embarassments, these are designated by numbers, stating only the states from which they come. In the endeavor to ascertain the different forms of meal made, this collection of samples must not be considered as representing the relative production, inasmuch as the very numerous small "custom mills" scattered throughout the rural districts generally make corn meal in one very simple manner, with a pair of burr-stones, driven slowly by water power.

The examination of these meals has been made as follows: and since in bread making, any husk is always removed, all was first passed through a sieve of sixteen meshes to the inch.

1st. *Volume*: 50 grms. of meal were jarred vertically, for 5 minutes, in a glass cylinder, 100 c.c. measuring 7 inches in height, and from the volume read off, the weight in pounds, of a bushel of meal was obtained.

2nd. *Size*: 100 grms. of meal was shaken uniformly for 10 minutes in a nest of eight brass sieves, which time was longer than necessary for most samples, but in a few instances, where the sample was more oily, the smaller sizes were not sharply separated. These sieves, to one inch, had meshes: 16, 20, 24, 30, 36, 40, 50. Any finer sizes would have been of no use. On Table I, the per cent passing each number of mesh is indicated, omitting fractions.

3rd. *Fats:* In some typical samples only, as indicated, a determination of the fat was made. Sudan III was used as a staining in examining the meal under the microscope and proved very satisfactory in bringing out the fat globules, but it afforded no quantitative estimation. A comparative estimate of the amount of freed fat was obtained by noting the length of time respectively taken by each specimen to attain the same (dark brown) color when soaking in a water solution of Osmic acid. About .25 grms of meal in a watch glass was moistened by 3 c.c. of a 0.2 per cent solution of OsO_5 in from 10 to 30 minutes or more, the uniform color was obtained. The comparison was made with three pieces of cardboard giving near shades of a brownish black color and the time was noted as the darkening sample passed each of these shades of color; the average of these three periods is the figure given on Table I.

And we may take the reciprocals of these figures as expressing the amounts of free fat present.

4th. *Cooking:* While it may be that for each variety of meal there is a mode of cooking to which it is specially adapted—in order to obtain a comparison of the meals, the method of cooking selected is of the simplest kind and one which conspicuously brings out any imperfections or flavors.

Each sample was treated approximately as follows:

A.	Meal, sifted	100 grms.
	Salt	2 "
	Water—about	90 "

Mixed quickly to a soft dough, formed to 3 or 4 small rolls, placed on a pan and baked in a hot oven, at 440 Fahr. for 30 minutes.

B.	Meal, sifted	100 grms.
	Salt	2 "
	Lard	10 "
	Water—about	85 "

Mix with salt, "lightly" mix with lard—then with water and bake as above.

One can expect little satisfaction from single tests of this kind not only because of the special difficulty of making ordinarily good bread upon so small a scale, and of baking it uniformly from day to day, but still more, in observing and expressing the slight differences of taste that are presented.

In describing the character of the bread, the following abbreviations are employed in the Table:

g, good; p, poor; f, flavor; n, no flavor; s, sweet;
c, coarse; t, tough; d, dry; v, very;
w, white; W, exceptionally white; y, yellow; Y brilliant yellow.

TABLE I.

Whole grain burr ground	Kilned or Raw	Bushel weighs lbs.	Sized by sieves mesh to inch							Required for color min.	Cooked	
			16	20	24	30	36	40	50		Color	Taste, etc.
(1) Va.	R	57	2	6	8	15	20	30	19	19	w	gd
(2) "	K	55	.3	2.	4.7	11	21	17	44	14	w	gd
(3) "	K	52	0	.1	.5	7.4	27	48	17	30	w	g
(4) "	K	59	0	1	13	20	19	36	11	14	w	gt
(5) "	R	55	1	3	6	12	22	32	24	17	w	vgfs
fat: 4.71												
(6) Tenn.	R	57	1.6	4.4	9	13	27	26	19	20	w	gf
(7) N. C.	R	56	.1	.6	3.3	14	20	35	27	22	w	vgf
(8) "	R	62	1	10	20	16	29	14	10	12	w	gfs
(9) Ala.	R	57	2	6	11	16	18	20	27	12	w	gst
fat: 4.39												
(10) Del.	R	60	0	0	.4	3.6	31	40	25	10	w	pt
(11) Ark.	R	60	1	5	16	17	29	17	15	7	w	gf
(12) Miss.	R	62	2	6	19	17	14	33	9	9	w	gs
Roller ground												
(13) Md.	K	55	0	1	2	19	20	41	17	25	w	gf
(14) "	K	54	0	0	1	14	22	42	21	29	w	gft
(15) Ky.	R	56	1	7	14	19	15	26	18	14	w	gcs
(16) "	R	52	1.3	4.4	7.3	16	18	34	19	40	w	gcs
Fat: 2.20												
(17) Mich.	R	59	0	3	13	18	26	21	19	29	y	gsf
(18) Iowa	R	54	.6	7.4	24.	27	26	11	4	48	y	scf
(19) Kan.	K	58	0	.1	.2	3.7	31	40	25	77	W	vgf
Fat: 1.78												
(20) Conn.	K	56	.2	8.6	36	27	16	9	3.2	108	Y	gcf
Fat: 1.75												
Degerminated roller ground												
(21) Pa.	R	55	1	1	6	18	22	29	23	45	y	gcf
(22) Ky.	K	52	0	0	1	13	42	28	16	15	w	vgn
(23) Mo.	K	54	.1	.1	5.8	17	30	27	20	33	W	gcn
(24) Mo.	K	59	0	0	6	23	36	19	16	29	W	gt
Fat: 1.25												

TABLE I.—*Continued*

Whole grain burr ground	Kilned or Raw	Bushel weighs lbs.	Sized by sieves mesh to inch							Required for color min.	Cooked	
			16	20	24	30	36	40	50		Color	Taste, etc.
(25) Iowa		59	0	5	17	20	23	19	16	28	W	gt
(26) Neb.		56	0	0	3	19	38	19	21	18	W	gtc
(27) Ill.	K	61	0	2	10	22	28	22	16	78	w	gn
(28) Mich.	R	53	0	.3	4.7	21	21	30	23	72	Y	gscf
Whole grain ground at Univ. Va.												
(29) Ill. Agr. Dept. fat: 5.66			1	4	14	11	37	23	10	46	w	gn
(30) Ill. Agr. Dept. fat: low ground			1	5	17	15	29	15	18	31	w	gnd
(31) Ill. Boone Co. once:			10	15	16	12	12	18	17	25	w	gc
(32) Ill. Boone Co. twice:			7	14	17	12	13	15	22	21	w	
(33) Ill. Boone Co. thrice:										20	w	

In order to compare the weights per bushel, it seemed well to employ a uniform method of settling the meal, hence it was jarred in the measure until it would settle no further. Specimens containing much of fine powder settle very compact, therefore in this respect these results cannot be compared with those usually observed.

It may be noted that some of the granular meals are compact but others as (16) and (22) which appear to be "cut meals" are more bulky.

Some years ago, it was the custom of many steam driven mills to grind corn by burr to a fine powder, in order to make a whiter meal, resembling wheat flour; this made a very compact bread. But no such meal is found among the samples examined, and the making of such meal seems to be now generally discontinued and replaced by the manufacture of a meal which is chiefly coarse and contains but little of powder. The superior whiteness of

some of these specimens is attained by selection of well-matured white corn, "scouring" the grain when shelled, and removing all husk and germ as soon as they are set free by the rollers employed—as seen in Nos. (19), (23), (25). Similarly from yellow grain very clean specimens are Nos. (20) and (21).

It is to be noted that the nutty flavor of white corn (somewhat like that of a chestnut), as well as the peculiar flavor of yellow corn are volatile bodies (i. e. odours) and are largely retained by the fat. The flavor is therefore to a large extent removed with the germ and is diminished by too much heating in the grinding or by Kiln drying. It is also removed by too long repeated grinding as is shown in No. 31 made by a single grinding of good grain in a metal hand grist mill, while the same grain was ground twice in making No. 32.

Nos. (29) and (30) were made of good corn and were run through the metal grist mill three times, thereby losing all "flavor." All the degerminated meals are found to be without the nutty flavor although No. (22) is certainly excellent in all other respects.

As to the kiln-dried meal of white corn, they too, generally retain little flavor, but Nos. (19) and (22) make excellent bread and have a pleasant sweet taste.

In examining this lot of selected samples, generally donated by the makers, it is natural that in tasting the raw meal I found but one that possessed any mustiness, and in this instance the sample was obtained from a retail grocery store without any inquiry as to its freshness.

The removal of the germ presents an important economical feature. Taking the whole meal as containing 5 per cent. fat, the degerminated meal will have about 1.5 per cent. fat, thus 100 lbs. of meal would so lose about 3.5 lbs. of edible digestible fat, which will correspond, allowing for the increase in starch, to a loss in food value of 30 cents or more. The reality of this loss is brought out by the fact that when cooking the meal, or other material to be eaten with it, one will ordinarily add this much additional fat, at a cost of 10 cents or more per pound, to replace that which has been removed.

As to the effect upon cooking.

The making of a proper dough largely depends upon the fine particles retaining water sufficient to fully soften the enclosed larger grains when heated in the oven, hot from the first. If the meal is too largely coarse it can hardly be made to stick together, and the dough presents a rough surface, from which the water may escape (dry out) before the starch and protein are properly softened, so producing a very hard bread, insufficiently cooked. An application of this principle is presented in a favorite method of cooking an "ash cake." The plain corn meal dough is wrapped in a cabbage leaf and buried in hot ashes until done. In this the flavor of the meal is peculiarly well preserved and we may obtain a very appetising food. Hence it is that some portion of the corn must be finely ground to obtain a meal for general use.

If a meal is too fine, it may become too compact when made into a dough, in such case the addition of a very little baking powder, say 3 grms. to the foregoing receipt will sufficiently open the dough to give good bread.

On the other hand very coarse and gritty meal is, in this respect, better adapted to making mush or a batter bread in which, while being cooked, it necessarily remains in contact with an excess of water.

All of the above samples of meal were also cooked according to receipt B. and there was little variation in the good bread obtained—tender, porous and appetising but varying with the peculiarities of the grain as to texture, color, and flavor of the meal.

The meal thus mixed with the fat should be but lightly pressed, with no pressing or "working" (as must also be done in making pastry with wheat flour), so subdividing the dough; and the resulting bread will be excellent.

We have in corn a natural mixture of starch, protein, fibre and fat, which, when simply ground and moistened by water, gives a dough all ready for baking, the oil serving to separate the mass and prevent it from becoming too compact or hard; hence it is to be treated in a wholly different manner from wheat and, to the writer, is not to be cooked with wheat flour with any advantage.

It seems therefore that the work done in refining corn meal is, so far as its use for bread is concerned, not well directed, in that the portion of the grain so removed is the very portion in which rests one of its chief advantages. It is true that meal from whole corn will not keep, under most conditions, more than 3 or 4 weeks, but is it not profitable to supply this fresh meal to obtain its several advantages?

In recent years there has been much and successful endeavor to free wheat flour from all bran and husk, so securing its pure whiteness while losing the advantages of the rougher fibre and ash content. But I think I have shown that similar processes do not improve corn meal, and that there is by this process loss of some of its most valuable constituents.

It appears from the favor with which oatmeal and many of the numerous breakfast foods are received (so much so, that in some cases these are sold at from 3 to 5 fold of the price of the grain from which they are made), that there is a craving of many stomachs for rougher food such as stimulates the processes of digestion; and this is certainly in many instances the explanation of the satisfaction with which corn bread is preferred by many as a staple diet.

But when corn is well matured, kept to thoroughly dry on the ear, and then, as it is needed, ground without heating, by burr stones, slowly turned by a water wheel, it furnishes a sweet, nutty flavored meal, which combines the most valuable of nutrients, and when cooked in the simplest manner, furnishes a food which is to many of mankind very acceptable, and to some, the staple of life.

(*Abstract:*)

FOOD STANDARDS, THEIR NATURE, HISTORY, AND FUNCTIONS

By William Frear
State College, Pa.

Nearly all civilized lands have enacted general food laws covering all foods. They prohibit the sale, as normal, of products that depart in certain, very generally defined ways from the normals corresponding to the food names used; but do not define these normals. In the absence of sufficiently complete, accurate, and concise definitions of these normals, the executive officers of these laws have been obliged to judge for themselves what the respective normals are, subject to the confirmation of the Courts.

A food standard is the expression of a food normal, and may include chemical and physical limits indicative of kind and quality. The existing systems of standards, British, Bavarian, German, Austrian, American, Swiss, Italian and Holland, are the work of experts representing the executive branches of the respective governments.

Nature: Food standards should correspond to the people's concepts corresponding to the several food names, and, since the laws with which they are to be used are quasi-criminal, should correspond to the lowest quality, within the kind, acceptable without notice of inferiority. They should represent present usage, the concepts of the public, where they differ from those of the trade, and those of home, instead of foreign countries. Since foods are chiefly of domestic, rather than of factory production, domestic usage should determine the normals, but due consideration should be given to the requirements of commercial distribution, as contrasted with immediate, domestic consumption.

Matter: As their material, food standards should contain what is necessary, (1) to distinguish the various normals from

each other; (2) to distinguish each normal from its substitutes, imitations, and adulterated modifications. The data should be both authentic and broadly representative. A standard may be useful, even though incomplete; but incompleteness of chemical and physical data should not lead to a definition of species broader than the public concept therefor. Such data should represent the products of the country in which the standard is to be used.

Consistency: Systems of food standards should be consistent; but the criterion of consistency is external, not internal; consistency with the people's concepts, not etymological consistency is the aim. Even a given word varies in its meaning with the context. Moreover, there is no simple mathematical formula that can serve safely to fix the relation of the minimum to the average of quality.

Form: A clear, concise definition, closely knit with the most serviceable limits, will better endure legal analysis than the encyclopediac form of description with loosely appended chemical limits. The latter form is, however, superior educationally.

Function: Standards are practically essential as bases of reference in the enforcement of general food laws, unify executive and judicial decisions, relieve the trade and the public from confusion and uncertainty. They do not interfere with variety in production, nor should minimum standards tend to lower the general average of excellence; since, however, such standards do not in reality represent the absolute minima of quality, their limits should be set with caution and common sense, and be widely published before application, lest honest, but unskilled or ignorant producers be injured.

International standards are practicable only for a limited number of products, but are desirable for these.

Provision should be made for the continuous addition of new limits and standards as the need and data therefor appear; but radical changes, made with frequency in existing standards, are gravely unsettling to producing interests.

THE PACKING OF AMERICAN SARDINES

By H. H. Hanson

Scattered along the Maine coast from Portland to Eastport there are about fifty-five different factories, whose combined annual output is usually somewhere between 125 and 200 millions of cans of sardines, valued at from five to seven million dollars according to the season. A large proportion of this industry is located around Eastport and Lubec, in Passamaquoddy Bay, where a majority of the inhabitants are dependent upon it, either directly or indirectly, for their livelihood.

A study, not yet entirely completed, was undertaken for the purpose of obtaining information on certain points of this industry which were perplexing alike to the packers themselves and to the food officials of the country. A comparison of the American and Foreign packed sardines and a description of the packing process is at once interesting and instructive as throwing light on some of the points under consideration.

The name sardine, which comes from the island of Sardinia, around which sardines abound, is not the name of a particular species, but is applied to fish of the genus *Clupea*, various species of which are canned in different parts of the world.

There are three important respects in which the Maine sardines differ from the foreign sardines, of which the French pack is generally recognized in this country as the most desirable. First, the fish packed in France under the name sardine is the *Clupea pilchardus*, while the fish packed in Maine under that name is the *Clupea harengus*, two distinct species of the same family which differ somewhat from each other both in appearance and flavor. Second, French sardines are packed in olive oil while the Maine sardines are put up in cottonseed oil. Third, in handling the French pack the single fish is the unit and quality is at all times considered of paramount importance; while in handling the Maine pack the hogshead is the unit and quantity is always sought. In America the French sardine retails for from

thirty-five to sixty cents per can, while the Maine sardine retails for the most part for five cents. The markets for these two general grades seem to be well established and although some fancy goods, which bring a high price, are put out it is quite certain that the Maine packers cannot be brought to the point of adopting French methods of handling even though the product might thus be improved in quality.

The fish are caught almost entirely in weirs, large circular or oval traps having long wings extending out from the one opening and arranged so as to guide the schools into it. These weirs are large enclosures built mainly of brush topped with marlin and are so located that there will be perhaps twenty feet of water in them at low tide. Fish may be taken coming in on the flood tide or going out on the ebb. It is not uncommon to take 100 hogsheads in a single weir, and even larger catches have at times been reported.

When a catch has been made the weir-man closes the gate of his trap and awaits the coming of the sardine boat. The competition between the boats running for the different factories is often very keen. Not many years ago there was sharp bidding between the skippers of these boats so that the prices paid for the fish were often ridiculously out of proportion to their value. As high as $30 per hogshead has been given. During the last few years a more or less fixed price has been paid for the fish and the boat which arrives first at the weir has first claim. At the beginning of the season about $12 per hogshead may be offered and later, when the fish become more plenty, the price is dropped to about $6.

When the fish have been purchased a purse seine or net is carried around the entire catch in the weir, drawn together at the ends, and closed at the bottom, so that the sardines are brougth into a compact mass, and may be scooped with hand nets into dories and conveyed to the sardine boats lying just outside. These boats range in size from remodelled life boats holding perhaps ten hogsheads to trim two-masters holding eighty hogsheads. As the fish are scooped into the holds of these boats salt is sprinkled over them, at the rate of about 100 pounds to the hogshead, and when the hold is full a race

for the factory is begun, each skipper striving to land his catch first. Arriving at the wharf of the factory the boat is drawn under a windlass from which a tub is let down to the hold and the fish are scooped into the tub, drawn up, and dumped into a sluice as fast as it is possible to work. Along this sluice, flushed with water, the fish run, sometimes through a contrivance which separates the large from the small, into large wooden pickling tanks where they are kept in salt brine long enough to give them a flavor and aid in preserving and hardening the flesh. Time allowed in the pickle varies with the condition of the fish and amount of salt they have already had in the boat.

From the tank most of the fish run to the flaking machine, a mechanical arrangement for distributing the fish evenly upon heavy wire screens upon which they go to the steam boxes for cooking. In some factories this flaking is done by hand, but if the machine is well made and properly run it will do the work much faster, although it is inevitable, especially when the fish are fat and tender, that many are broken. From the steam boxes the fish are run into a drier where they are kept long enough to thoroughly dry and, upon emerging from this process, and sufficient time having elapsed for them to cool, they are taken at once to the packing tables. The packers are girls and women of all ages who cut off the heads with shears and pack the fish in cans so rapidly that although they are paid only about sixteen cents per case of one hundred cans, they earn at times three or four dollars a day.

The small fish are for the most part packed in oil in small cans holding four ounces. These are called "quarter oils." The larger fish are usually packed in mustard sauce in cans holding eleven ounces. These are called "three-quarter mustards."

Usually the oil is placed in the can after the fish but the mustard sauce is put into the cans first. After the cans are filled they are taken to the sealing machine and when the covers are in position they are processed in a tank of boiling water from one and a half to two and a half hours. This process is called bathing. They are then cooled, cleaned, examined for leaks, "fats" and "slacks," and finally packed in cases for shipment.

"Fats" are cans which have been carelessly filled too full and, for that reason might afterwards be mistaken for "swells," which have spoiled by fermentation. Such cans are punched and oil drawn out to reduce the swelling, after which they are again sealed and bathed. "Slacks" are those which do not contain enough and these are punched, heated and oil drawn in to fill them out.

There are a number of modifications of this general process in use in various places. A few packers behead all fish before cooking. A few fish are fried or baked instead of being steamed. Some of the quarter-oils contain a bay leaf or a clove and there are a few fish put up in tomato sauce. Several different styles of ovens and drying machines are in use and there are many different types of sealing machines, a new one now being perfected apparently being a great improvement over any other previously made. Whereas all other machines now in use seal the cans by rolling the edges of the can and cover tightly together, the new machine seals them hermetically. This machine also automatically introduces into the cans the proper amount of oil, fluxes the edges, places the covers and, after tightly sealing, turns them out at the end ready for the bath at nearly double the rate of the old machines. An endless belt is arranged to bring the cans to this machine directly from the packing tables.

There is always great waste of fish during the process. A twenty per cent loss of the catch is always reckoned upon and it sometimes runs as high as fifty per cent. When fish break in large numbers during the process of preparation for the cans as happens under certain conditions, the waste is large. When large fish which would ordinarily go as "mustards" are cut to the size of "quarter oils" the waste is again large for the fish are sometimes cut in two in the middle in order to make them short enough for the purpose. This latter practice is resorted to, however, only when particular orders for "quarter oils" must be filled regardless of the size of the fish. An attempt to utilize some of this waste is being made in some factories by canning it as "deviled fish," but most of the waste is sold at a dollar and a half per hogshead to the fertilizer factories where the oil is pressed out and the pomace used in fertilizer.

The two most important questions which have been studied are the cause of swells, that is, cans swelled out by inside pressure caused by fermentation, and the breaking of the fish during the process of preparation for the cans.

By correspondence with packers and by investigation in the factories it seems evident that the cause of the swells is imperfect sealing rather than imperfect or incomplete processing or bathing. Experiments with four-ounce cans ("quarter oils") and with eleven ounce cans ("three-quarter mustards") were made at several factories and it was found that in from twenty-five to thirty minutes after the cans were immersed in the boiling bath the temperature had risen at the middle of the can to 100 degrees C. The smaller cans are bathed in the various factories from one and a quarter to two hours, and the larger cans from one and a half to three hours, the time varying in the different factories and with different conditions of the different catches. This would seem to be long enough to thoroughly sterilize the contents of the can. That this time is sufficient for such sterilization is indicated by the fact that in all cases where the hermetically sealed cans are now put out there is practically no complaint from swelled cans. This fact, taken in connection with the other fact that with the ordinary roll sealed cans leaks often occur and are found even before the goods have left the factory, would seem to place the blame for the swelled cans almost entirely upon the sealing machines which do not thoroughly close the cans. Corroborative evidence is furnished by the further fact that practically no living organisms were found on opening thoroughly sealed cans.

There are at least six different causes contributing to the breaking of the fish during the process of preparation for the market.

First. Fish that have lain in a shallow bay or over mud flats for several days will have softer flesh than those taken from deeper, cooler water, and are, of course, much more easily broken.

Second. Fish which are very fat are naturally more tender than fish which are lean and will break more easily, especially if they are not properly salted.

Third. The rough, careless handling which many of the fish receive would break even the firmest fish before they were finally placed in the cans.

Fourth. If fish are over salted they tend to break transversely across the side after steaming and drying.

Fifth. When the fish have undigested food in them softening and breaking commences quicker than otherwise.

Sixth. When the fish are kept too long out of water without sufficient salt, as is sometimes the case, they naturally begin to soften and decompose.

It has appeared to some that the breaking of sardines indicates that they are unfit for food. That this is not so is evident from the above. Of the six reasons given for the breaking only the two last could be considered as evidence of unfitness for food, and that the first of these is sufficient evidence of unfitness is questioned. That those which have burst from the last cause are unfit for food is, of course, unquestioned and, as their unfitness is at once made evident by the odor, they should be sent to the fertilizer factory. The breaks which occur in these fish are somewhat characteristic of the cause of the breaking. For example, as above stated, over salted fish after steaming and drying tend to break transversely along one side; fat fish tend to break along the backbone where the fat is deposited thickly and the skin is tender; feedy fish, that is fish with undigested food in them, soften very rapidly and tend to break along the under side where the flesh is thinnest. Rough and careless handling increases the tendency to break in all the other cases. That the breaking along the under side of feedy fish does not necessarily indicate unfitness for food would appear from the fact that regardless of thorough salting and careful handling the breaks begin to appear in about three hours after the fish are taken out of the water, but fish so breaking give no evidence whatever of either decomposition or decay, are as sweet and palatable, and, aside from appearance seem as good as those not broken.

Feedy fish, above mentioned, are usually full of either one or the other of two kinds of food known to the fishermen as "shrimp" and "red feed." This latter is the bane of the sardine industry

as it has long been known that fish containing it deteriorate much more rapidly than fish in any other condition. They often begin to break open before reaching the factory, and, if they contain much of this material, by the time they are ready for the can they are broken so badly that a large percentage of the catch is entirely unfit in appearance for packing. The study of the subject of breaking involved the questions: "What is 'red feed'? Why does it cause this deterioration?"

This "red feed" we have identified as one of the copepods, *Temora longicornis*, a microscopic crustacean of the family *Centropagidæ*. It is quite abundant in the region of Woods Hole, Mass., during the winter months and is described by Dr. William Morton Wheeler in a bulletin of the United States Fish Commission for 1900. Dr. Wheeler speaks of it as an essentially boreal form rarely seen in the above locality during the months of July and August. These are the months in which it is most abundant along the coast of Maine. Dr. F. H. Moore of the United States Fish Commission in a report of his investigations in the herring fisheries of Maine some years ago speaks of this crustacean as one of the copepods but apparently the species was not determined. He calls it in his bulletin "red seed" but this is evidently an error either in printing or in information.

It has long been recognized by sardine canners that fish containing "red feed" deteriorate much more rapidly than those containing any other kind of food but why this is so no one to our knowledge has ever before attempted to determine.

Althougth the sardines were never more abundant on our coast than during the season just passed, "red feed" was not particularly troublesome, so that not enough was obtained upon which to make thorough investigations, but two possible answers to the second question, the cause of the rapid deterioration, have suggested themselves. First, that the deterioration is due to auto-digestion induced by some particular enzyme. Second, and this seems more likely in the light of present knowledge, that a methylamine is responsible for the trouble. This compound has been identified in various fish and crustaceans. It was reported many years ago as being present in the roe of herring and it is not impossible that in this case "red feed" was

mistaken for roe. It has been reported very recently by Bigelow and Bacon as being present in considerable amount in the shrimps which are canned so extensively in Mississippi. In their investigation of this problem which was reported in the Journal of Industrial and Engineering Chemistry for November 1911, it was noted that this compound affected the hands of the workmen and had a corrosive effect upon materials which came in contact with the shrimp containing it. A parallel case seems to be found in the sardine industry, for at times when "red feed" is abundant the hands of the operatives who work upon the fish are made sore and at times also a strong odor of ammonia is noted during some of the processes. Either auto-digestion produced by enzymic action, or the presence of a methylamine, would probably cause the rapid breaking down of the tissues and the consequent softening and breaking of the sardines containing "red feed" and it is hoped soon to further investigate the question.

In closing it should be stated that the credit for a large part of the foregoing should be given to A. M. Buswell, Instructor in Industrial Chemistry in the University of Maine, who acted as field agent during the summer of 1911, and thanks are also due Dr. O. A. Johannsen of the Maine Experiment Station for aid in identifying the "red feed."

Maine Agricultural Experiment Station,
Orono, Me., U. S. A.

ETUDE CHIMIQUE DES FRUITS DE SORINDEIA OLEOSA

Par M. Alexandre Hébert
Paris, France

I.—La matière première de cette étude consistait en fruits séchés au soleil de Sorindeia Oleosa A. Chev. qui nous avaient été adressés par M. Auguste Chevalier et qui provenaient d'un arbre commun au Soudan. Ce sont des fruits à noyau entouré de pulpe et de la grosseur d'une cerise; ils ont deux usages et ont été examinés à deux points de vue:

1°—La pulpe ou péricarpe du fruit est très sucrée; dans le pays d'origine, on fait fermenter ces fruits pour en obtenir une boisson analogue au cidre; 2° l'amande de la graine proprement dite, qui forme le noyau du fruit, est très oléagineuse; on en extrait de l'huile et on en prépare du savon.

Il convenait donc de vérifier, d'une part, la nature et la proportion du sucre existant dans la pulpe du fruit; d'autre part, la quantité et les propriétés de la matière grasse contenue dans les amandes.

II.—Pour effectuer l'étude chimique de ces fruits aux points de vue qui nous intéressaient, nous avons commencé par séparer les pulpes et les noyaux. A cet effet, 500 grammes de ces fruits séchés ont été mis en contact avec une quantité d'eau froide suffisante pour les recouvrir, après 24 heures de séjour, ils s'étaient gonflés et étaient d'une consistance telle qu'ils pouvaient être malaxés dans l'eau sans risquer d'écraser les noyaux. Ceux-ci, séparés ainsi des pulpes, ont été desséchés à l'air et mis de côté pour un examen ultérieur. On les a trouvés en proportion de 40 pour 100 des fruits secs accusant ainsi 60 pour 100 de pulpes.

Les pulpes gonflées ont été épuisées à trois reprises par l'eau froide pour dissoudre toutes les matiéres solubles et notamment les sucres qui s'y trouvaient. Finalement le résidu a été pressé

et le liquide provenant de ce pressurage a été joint aux liqueurs d'épuisement. Celles-ci ont été déféquées par le sous-acétate de plomb et le liquide filtré a été débarrassé de l'excès de plomb par l'hydrogène sulfuré. La solution incolore ainsi obtenue a été concentrée dans le vide au bain-marie à très basse température jusqu'a consistance sirupeuse, puis abandonnée à elle-même. Elle a refusé de cristalliser, malgré tous les subterfuges habituels employés dans ce but: concentrations diverses, reprises par l'alcool traitement au noir animal, etc. Le sirop réduisait énergiquement la liqueur de Fehling, donnait avec l'acétate de phényl-hydrajine une osazone cristallisée en aiguilles groupées en forme d'éventail, fusibles à 200° et correspondant aux propriétés de la phényl-glucosazone, déviait enfin à gauche le plan de polarisation de la lumiére, mais cette déviation correspondait à une quantité de sucre réducteur bien plus faible que celle indiquée par le titrage à la liqueur de Fehling. Somme toute, ces caractères répondaient au sucre interverti.

D'autre part, on a trouvé dans une quantité donnée des fruits secs, épuisés par l'eau froide comme nous l'avons indiqué, et par titrage à la liqueur de Fehling, une proportion de 22 pour 100 de sucres réducteurs et une quantité nulle de sucres non réducteurs. Si nous admettons dans ces fruits, à l'état frais, une teneur en eau égale à 90 ou 95 pour 100, teneur qu'on retrouve généralment dans les fruits de ce genre, la proportion de sucres réducteurs correspondrait à 1:10 ou 2:20 pour 100 des mêmes fruits à l'état frais.

Il résulterait de ces expériences que les matières sucrées des fruits de *Sorindeia Oleosa* A Chev. seraient constituées par du sucre interverti, mélange de glucose et de lévulose, ce qui justifierait leur emploi indigène pour la préparation d'une boisson plus ou moins alcoolique, et du genre du cidre, mais qui, en tous cas, ne peut certainment être que très peu riche en alcool.

III.—Les noyaux, obtenus comme nous l'avons dit, et qui constituaient 40 pour 100 des fruits secs, renferment 24 pour 100 de ces mêmes fruits secs en amandes. Celles-ci, après broyage et extraction à la benzine, lui abandonment une matière grasse dont la proportion atteient 25 pour 100 des fruits secs.

La matière grasse obtenue est solide à la température ordinaire, de couleur brunâtre et présente les constantes suivantes:

Densité à 17°	0.889
Point de fusion	16–17°
Point de congélation	12–13°
Indice d'acidité	4.90
Indice de saponification	185.00
Indice de Reichert	7.92
Indice d'Hehner	91.75
Indice d'iode	132.00

La graisse de *Sorindeia Oleosa* A. Chev., saponifiée par la soude alcoolique et acidifiée, fournit 92 pour 100 environ d'acides gras, jaunâtres, solides à la température ordinaire, fusibles à 39–40°. La séparation des acides gras saturés et incomplets effectuée par l'épuisement à l'éther des sels de plomb, a donné 24 pour 100 d'acides incomplets, liquides, de couleur jaune brunâtre, et 76 pour 100 d'acides saturés, solides, colorés en jaune brun, fondant à 44–45°. Ce point de fusion assez bas indique l'existence, dans la graisse étudiée, d'acides gras relativement inférieurs. L'usage de la graisse de *Sorindeia Oleosa* A. Chev. pour la préparation du savon se comprend ainsi parfaitement, cette substance grasse d'une part, ne paraissant pas comestible, et d'autre part, donnant des acides gras à point de fusion trop bas pour servir à la fabrication de bougies ou même de chandelles.

SUR LA COMPOSITION DE DIVERS PRODUITS, GRAINES OU TUBERCULES AMYLACES OU FECULENTS DE L'AFRIQUE OCCIDENTALE FRANÇAISE

Par M. Alexandre Hébert
Paris, France

Au cours de sa dernière mission en Afrique occidentale française, M. Aug. Chevalier a rapporté un certain nombre de produits, graines ou tubercules, de nature amylacée ou féculente, qu'il nous a remis pour en déterminer la composition chimique, en apprécier la valeur nutritive et en fixer l'emploi industriel possible. Ce sont ces diverses recherches que nous résumons ici.

GRAINES.—Maïs blanc du Dahomey.—Ce maïs nous a été remis sous forme d'épis dont une certaine quantité étaient malheureusement charançonnés; nous avons pu cependant en trouver quelques-uns intacts sur lesquels nous avons effectué l'analyse. Nous avons séparé dans les épis les glumes et glumelles, les rachis et les graines dont nous avons déterminé la composition. Nos dosages nous ont conduit aux résultats suivants:

	Séchés à l'air	*Séchés à* 110°
Poids moyen d'un épi entier	120 gr.	110 gr.
Décomposable en glumes et glumelles	16.6	16.6
graines	88.3	78.3
rachis	15.0	15.0

Analyse de la graine séchée et moulue

Humidité restant	1.63%	
Matières minérales	1.96	dont 0.49 solubles dans l'eau.
Matières grasses	3.70	
Matières azotées	11.55	dont 0.98 solubles dans l'eau.
Sucres réducteurs	0.36	
Sucres non réducteurs	0.95	
Gommes, tannins, acides végétaux	0.24	
Amidon	76.30	
Cellulose	1.36	
Vasculose	1.90	
Total	99.95	

Cette graine peut donc être comparée, au point de vue de sa valeur, à nos produits indigènes. Elle est d'autant plus intéressante qu'il s'en exporte d'Afrique des quantités importantes dont l'introduction pourrait rendre service à diverses industries.

Voandzeia Poissonni A. Chev.—Ces graines qui proviennent d'Ouaga dougou (Mossi), présentaient la composition ci-dessous.

Humidité	10.38
Matières minérales	4.34
Matières grasses	1.91
Matières azotées	21.41
Sucres réducteurs	traces
Sucres non réducteurs	0.41
Amidon	48.77
Cellulose	12.74
Total	99.96

Cette graine, riche en matières azotées, renferme moins d'amidon que les graines amylacées de nos pays; elle peut néanmoins être employée au point de vue alimentaire au moins dans les contrées d'origine.

TUBERCULES.—Ignames.—Ces tubercules qui nous ont été envoyés à l'état desséché, provenaient de la cote d'Ivoire; ils ont donné à l'analyse les résultats suivants:

Humidité	13.80
Matières minérales	2.40
Matières grasses	0.40
Matières azotées	5.75
Sucres réducteurs	1.00
Sucres non réducteurs	1.00
Amidon	73.80
Cellulose	1.25
Vasculose	0.60
Total	100.00

Ces tubercules sont assez comparables comme composition à la pomme de terre. Ceux qui ont été expédiés en Europe ont

été trouvés de valeur au moins égale au manioc sec. Le commerce des ignames africains pourrait donc prendre de l'extension comme produit alimentaire sous une forme quelconque.

DIEGEMTENGUERE (Vulg. Mossi).—Les tubercules de cette plante qui nous ont été remis provenaient d'Ouagadougou dans le Soudan français. Leur poids avait été déterminé à l'état frais ce qui nous a permis de fixer leur composition exacte à l'état frais et à l'état sec:

	Etat frais	Etat sec
Eau	57.90%	0.00%
Matières minérales	2.02	4.80
Matières grasses	0.21	0.50
Matières azotées	4.47	10.62
Sucres réducteurs	Néant	Néant
Sucres non réducteurs	2.69	6.40
Amidon	28.80	68.40
Cellulose	3.85	9.15
	99.94	99.87

Cette composition ratifie parfaitement l'emploi de cette plante qui est cultivée au Mossi, dans la boucle du Niger, pour ses tubercules alimentaires.

MOELLE d'ENCEPHALARTOS BARTERI.—Ce produit est extrait d'une plante de la famille des Cycadacées, dont la tige est pourvue d'une moelle abondante qui possède la composition suivante:

Humidité	12.80
Matières minérales	2.80
Matières grasses	0.60
Matières azotées	6.43
Sucres réducteurs	10.00
Sucres non réducteurs	1.10
Amidon	60.52
Cellulose	4.25
Vasculose	1.50
Total	100.00

Cette moelle est, comme on le voit, riche surtout en hydrates de carbone: sucres et amidon; cette richesse justifie parfaitement l'emploi indigène que l'on fait de cette moelle en fabriquant une sorte de pain avec la fécule qu'on en extrait.

(Abstract)

ON THE TASTE OF THE SALT OF GLUTAMIC ACID

KIKUNAË IKEDA

College of Science, Imperial University of Tokyo, Tokyo, Japan

The glutamates having the general formula $C_5H_8NO_4M'$ are mostly soluble in water and all of them have a very distinct peculiar taste, the quality of which differs from all other well defined taste qualities hitherto known. Numerous food materials present this taste, but so much overshadowed by others, that no clear conception of this quality has hitherto been formed, although it contributes largely to the flavor. For this taste quality the name "glutamic taste" is proposed.

This taste is then demonstrated to be that of the monovalent glutamate ion $C_5H_8NO'_4$. For this purpose the threshold value of the taste has been carefully measured for the salts of sodium, potassium, magnesium, calcium and barium. The value has been found to be $\frac{1}{2500}$ normal for all the five salts. The taste-imparting power of the glutamates is very great.

The author was led to the discovery of the taste of glutamates by his investigation on the constituents of a certain sea-weed, which is used in Japan as a flavoring. He isolated glutamic acid from it and found that it is the salts of this acid that give the weed its peculiar flavor.

There are numerous flavoring substances which give glutamic taste, and among them meat-extract and allied preparations. But from obvious reasons a pure glutamate is much to be preferred over them. Of all the glutamates of non-poisonous metallic radicals the sodium salt is the most suitable. Within the last three years the manufacture of this salt has arisen in Japan and it is now rapidly becoming an article of general consumption.

There is hardly any doubt that the glutamate will come to be manufactured in a large scale in Europe and America. As the raw material for the manufacture is the hydrolytic products of proteins, there is a prospect that the chemical industry of these products will be greatly developed, bringing in its train numerous problems of great interest.

PROGRESS REPORT OF NUTRITION INVESTIGATIONS IN THE UNITED STATES

By C. F. Langworthy, Ph.D.

Office of Experiment Stations, Department of Agriculture

Introduction

For many years, continuous progress has been made in the United States in the study of various questions concerned with human nutrition. In this summary, the attempt has been made to bring together articles on this subject which have appeared in the United States, since the 7th International Congress of Applied Chemistry, thus supplementing a paper of similar scope presented at the 7th Congress.

A survey of the literature under consideration shows that a considerable part of it represents work carried on under governmental or institutional auspices, a considerable part representing the work of the United States Department of Agriculture and other branches of the general Government and the agricultural experiment stations. University laboratories and the laboratories of endowed institutions are also large contributors as are also state boards of health.

In general, it may be said that judging by the amount of work which is published annually, interest in the experimental study of human nutrition is growing very rapidly. The fact is recognized that the record of work here presented is by no means complete but it is believed that it is sufficiently extended to show the character and scope of the work which is being done.

For convenience the material has been arranged under the following heads: Studies of Food and Food Products; Special Studies of Ash, Protein, and Other Food Constituents; Cooking in Its Relation to Nutritive Value; Canning, Preserving, Handling and Storage; Dietary Studies and Dietetics; Digestion; Metabolism; Respiration Calorimeters, Bomb Calorimeters, and Experiments with Them; Foods and Their Relation to Problem of Hygiene; and Cost of Living and Other Statistical Data.

STUDIES OF FOOD AND FOOD PRODUCTS.

As is usually the case, considerable attention has been given to the proximate composition of food products and to the effect of various processes of manufacture or handling upon nutritive value and quality. Many hundreds of proximate analyses, more or less complete in character, have accumulated during the periods under consideration, in connection with inspection work under national and state pure food laws and as a part of other work undertaken for some special purpose aside from analysis.

Many milling and baking tests with different varieties of wheat have been reported, this question being one which is of great interest particularly in wheat-growing regions. E. F. Ladd and Emily E. May (North Dakota Sta. Spec. Buls. 19, pp. 105–114; 24, pp. 179–194, fig. 1) have carried on extensive work of this sort with durum wheat flour. Their studies showed that more power was required to grind durum than Fife or Bluestem but the yield was as large and the bread made equal to that produced from other flours, though not quite so white in color. It was found to hold moisture better than that from commercial flours. A study of milling and baking of durum wheat flour was reported by L. M. Thomas of the North Dakota Experiment Station.

The effect of climatic conditions on the composition of durum wheat has been discussed on the basis of a number of analytical and other studies carried on by the Department of Agriculture, by J. A. LeClerc (U. S. Dept. Agr. Year Book, 1906, pp. 199–212, pls. 2.) Several hundred analyses of spring and winter wheat of different varieties grown in different States showed an average protein content of 12.2 per cent as compared with 14.7 per cent for over 100 samples of durum wheat analyzed by the author.

E. F. Ladd and C. H. Bailey (North Dakota Sta. Buls. 89, pp. 14–80; 93, pp. 204–253, dgms. 5) have reported an extended study of the milling quality of wheats of different varieties and crops.

Similar tests of wheat of different kinds and crops grown in different localities have also been made by R. W. Thatcher (Washington Sta. Bul. 84, pp. 48, figs. 3) and L. R. Waldron (North Dakota Sta., Rpt. Dickinson Substa. 1910, pp. 43, 44),

and with California wheats by G. W. Shaw and A. J. Gaumnitz (California Sta. Bul. 212, pp. 315–394, figs. 18, dgms. 3), and with a variety of wheats by F. D. Gardner (Roller Mill, 28 (1909), No. 5, pp. 201–204).

It is interesting to note the discussion of the future wheat supply of the United States by M. A. Carleton (U. S. Dept. Agr. Yearbook 1909, pp. 259–272, figs. 2), which is based on a digest of statistical data.

With respect to the effect of soaking and germination of wheat on the distribution and yield of milling products, the quality of flour, and bread-making properties, G. A. Olson (Amer. Food Jour., 6 (1911), No. 4, pp. 36–39, figs. 4) found that water-soaked wheat is not necessarily spoiled and can be used for milling purposes, providing it has been thoroughly cleaned and dried. Using small quantities of germinated wheat flour with other flour increased the volume of the loaf, according to the author, without impairing its texture. Each particular flour required a different amount of germinated flour to produce the best results. Too large an amount of diastatic flour is less beneficial than none.

Analyses of a number of sorts of gluten flour manufactured in the United States and of foreign diabetic products were reported in comparison with wheat flour by D. W. Fetterolf (Univ. Penn. Med. Bul., 22 (1909), No. 7, pp. 217–222).

From an experimental study of the starch grain, H. Kraemer (Amer. Jour. Pharm., 79 (1907), pp. 217–229, pl. 1, figs. 3) concludes that "the starch grain consists of colloidal and crystalloidal substances, these being arranged for the most part in distinct and separate lamellæ, that is, at the point of origin of growth and in the alternate lamellæ the colloidal substance preponderates, associated with the crystalloid cellulose; whereas in the other layers the crystalloidal substance, consisting for the most part of granulose, occurs in greater proportion."

Several studies of cane sugar and maple sugar have been carried on.

C. A. Browne, Jr., and R. E. Blouin (Louisiana Stas. Bul. 91, pp. 103) have summarized a large amount of data collected during recent years by the Louisiana Sugar Experiment Station, which have to do with the composition of the stalk, seed, root,

and leaves of sugar cane and of the plant ash. The work as a whole is an exhaustive study of the chemical composition of sugar cane, with reference to its use for sugar making, and of the physiology of the growth and ripening of the cane. Experimental work on sugar making is also reported.

From a study of the question of the influence of micro-organisms upon the quality of maple sirup, H. A. Edson (Abs. in Science, n. ser., 31 (1910), No. 791, p. 308) was able to show by isolation and inoculation experiments that to certain groups of micro-organisms is ascribable the abnormal type of sap of the late runs characterized by green, red, milky, and stringy appearance.

A. H. Bryan (U. S. Dept. Agr., Bur. Chem. Bul. 134, pp. 110, pl. 1, figs. 4, map 1), in connection with a study of maple sap sirup, reports analyses of 481 samples of sirup of known purity collected in maple-producing States in the United States and in Canada, the data being gathered as a basis for comparing and grading maple sirups.

Considering the 395 samples from the United States, the average moisture content was 34.19, sucrose 62.64, invert sugar 1.49, ash 0.66, and undetermined material 1.02 per cent. The polarization values were: Direct, at 20°C.,+60.93; and invert, at 20°C.,−22.16. The average values for the 86 Canadian samples were: Moisture content 34.34, sucrose 62.24, invert sugar 1.41, ash 0.62, and undetermined material 1.59 per cent. The polarization values were: Direct, at 20°C.,+59.33; and invert, at 20°C.,−23.17.

The results of a special study of the constituents of maple-sirup ash are also reported. The average results for 100 samples from different States showed that the ash contained 38.07 per cent potash, 21.88 per cent lime, 5.39 per cent phosphoric acid, and 1.59 per cent sulphates.

Considering the samples from both the United States and Canada, the average basic lead value of 2.70, calculated to dry substance, and the average neutral lead number was 0.79. The average malic acid value determined by the modified calcium chloride method was 0.84, and by the calcium acetate method, 1.01.

Factors which influence the character of the sap and the sirup and related questions are discussed.

Experimental work carried on in an attempt to isolate flavoring substances present in maple sap is described by A. P. Sy (Jour. Franklin Inst., 166 (1908), pp. 249–280); Abs. in Chem. Abs., 2 (1908), No. 24, p. 3376), in a publication dealing with history, manufacture, and analysis of maple products, and work reported on the analysis of maple products.

Housekeepers and manufacturers of food products often express the opinion that there is a difference in the culinary quality of cane sugar and beet sugar. The matter was studied by G. W. Shaw (California Sta. Circ. 33, p. 4), of the California Experiment Station. The sugar is being used for sirup making, for canning fruit, and for jelly making. The beet sugar produced more froth in making sirup, but investigation led to the conclusion that this was due to the finer granulation of the beet sugar, which caused more air to become entangled during the starting than was the case with cane sugar. No differences were observed in the keeping quality of canned goods or the jelly made with the two sorts of sugar from his experimental data and other evidence the author concludes that under commercial and household conditions, beet sugar and cane sugar give equally satisfactory results for these uses.

An exhaustive study was made of the composition of American honeys by C. A. Browne (U. S. Dept. Agr., Bur. Chem. Bul. 110, pp. 1–69, 89–93, pl. 1, fig. 1), of the Bureau of Chemistry, and a microscopical study of honey pollen by W. J. Young (U. S. Dept. Agr., Bur. Chem. Bul. 110, pp. 70–88, pls. 5).

A number of studies of meat, eggs, cheese, and other animal foods have appeared.

The glycogen content of beef flesh and the factors which influence it were studied, in animals recently slaughtered, by P. F. Trowbridge and C. K. Francis (Jour. Indus. and Engin. Chem., 2 (1910), No. 1, pp. 21–24). The length of time which elapses after feeding before the animal is slaughtered, the authors consider an important factor in determining the amount of glycogen which remains stored in the organs and muscles. Their results indicate that there is a rapid enzymatic hydrolysis of glycogen

in flesh under many conditions, but that at 10°C. or lower, it did not take place appreciably.

In connection with an extended study of market classes and grades of meat, L. D. Hall (Illinois Sta. Bul. 147, pp. 147–290, figs. 75; Abstract, pp. 15, figs. 4) has described and illustrated by diagrams or figures the standard grades of beef, veal, mutton, and pork as they are found in the Chicago wholesale trade. Technical terms are defined. The bulletin as a whole furnishes a large amount of data on the subject which is of importance in discussing meat in relation to dietetics as well as for other purposes.

Some data of a similar character have been published by P. F. Trowbridge (Missouri Bd. Agr. Mo. Bul., 9 (1911), No. 2, pp. 69–78).

W. D. Richardson (Jour. Amer. Chem. Soc. 29 (1907), No. 12, pp. 1757–1767) reports the results of the examination of a large number of samples of animal and vegetable foods with a view to securing data regarding the occurrence of nitrates in vegetable foods, cured meats, and elsewhere. He concludes that nitrates are quite generally distributed.

F. C. Cook (U. S. Dept. Agr., Bur. Chem. Ciro. 62, pp. 7), of the Bureau of Chemistry of the Department of Agriculture, has reported a large number of analyses of beef extracts and yeast extracts of known origin. According to the author, "the yeast extracts contain approximately 1 per cent ether-soluble material and the beef extracts larger amounts. Cholesterol was not found in the ether extracts, and sarcolactic acid only in the yeast extracts.

"The phosphorus of beef is largely water-soluble, consequently a considerable percentage of the ash of beef extracts is composed of this constituent. Approximately one half of the sulphur of beef is water-soluble. Yeast extracts derived from yeast rich in phosphorus also contain a large amount in the ash. The total amount present is larger than the ash content, showing that some phosphoric acid is volatilized on ashing. The organic phosphorus determined by the Siegfried-Singewald method gives approximately the 1:10 ratio compared with the total as suggested by those authors.

"The total nitrogen of the beef extracts on the water-free and fat-free basis averages 11.82 per cent, that of the yeast extracts averages 7.44 per cent. The amino nitrogen figures for the beef preparations are nearly double those of the yeast extracts.

"Although the water-soluble nitrogen of beef, which constitutes 25 per cent of the total nitrogen, consists of approximately two thirds and one third amino nitrogen, the samples of beef extracts analyzed average 72 per cent of amino nitrogen and 28 per cent of protein nitrogen.

"The general appearance and odor of the two varieties of extracts are very similar. As a food both are extremely limited in value. The beef extracts contain more nitrogenous extractives than the yeast preparations, otherwise their general composition is much the same."

A large number of analyses of samples of meat extract, meat juices, yeast extracts, and similar goods are reported and discussed by W. D. Bigelow and F. C. Cook (U.S. Dept. Agr., Bur. Chem. Bul. 114, pp. 7–56), the methods followed being described.

Meat extracts, yeast extracts, and similar goods were also tudised by J. P. Street, et. al. (Connecticut State Sta. Rpt. 1907–8, pt. 9, pp. 573–716).

On the basis of numerous tests, I. A. Field (U.S. Dept. Com. and Labor, Bur. Fisheries Bul., 28 (1908), pt. 1, pp. 243–257; Doc. 655, 1910, pp. 243–257) reaches the conclusion that the common sea mussel (*Mytilus edulis*) is nutritious, palatable, and easily digested. From tests of culinary qualities, made under a variety of conditions, of the smooth and horned dogfish, he concludes further that the flesh of these fishes is cheap, palatable, nutritious, and easily preserved, and he believes further that it is as digestible as that of other fishes.

In a paper on unutilized fishes and their relation to the fishing industries, I. A. Field (U. S. Dept. Com. and Labor, Bur. Fisheries Doc. 622, pp. 50, pl. 1) discusses methods of profitably using dogfish of different sorts, sand shark, toad-fish, etc., summarizes data regarding the use of fresh, canned and dried dogfish, and gives some results of tests of its culinary quality, which he believes indicate that such dogfish flesh is both palatable and wholesome.

The uniformity with which copper was found in oysters examined by J. T. Willard (Jour. Amer. Chem. Soc., 30 (1908), No. 5, pp. 902–904) led him to conclude that it is to be regarded as a normal constituent.

J. T. Willard and R. H. Shaw (Kansas Sta. Bul. 159, pp. 143–177) analysed all the eggs laid in 6 weeks by 4 lots of pure-bred chickens. On an average the thickness of the shells was 0.0139 in. In addition to usual determinations, they report data regarding the percentage of phosphoric acid, the ash in the yolk, and the ratio of phosphoric acid to ash. The average amount of ash was 1.57 per cent and of phosphoric acid 1.43 per cent, the ratio of phosphoric acid to ash being 1:1.09.

"It is evident that the ash consists almost entirely of phosphoric acid. This is doubtless produced almost entirely, if not altogether, from the lecithin of the egg yolk."

Mary E. Pennington (Jour. Biol. Chem., 7 (1910), No. 2, pp. 109–132) has reported the results of an extended chemical and bacteriological study of fresh eggs, which was reported at the London Congress of Applied Chemistry, in June, 1909, and later published in full.

L. L. Van Slyke and A. W. Bosworth (New York State Sta. Tech. Bul. 4, pp. 1–16, 17–22) at the New York State Station have studied some of the early chemical changes which take place in the proteids and in the calcium and phosphoric acid compounds of Cheddar cheese, and also the acidity of the water extract of Cheddar cheese.

A. W. Bosworth (New York State Sta. Tech. Bul. 5, pp. 23–39) has also reported the results of chemical studies of Camembert cheese.

The manufacture of a food product called buttermilk is described by J. L. Sammis (Wisconsin Sta. Bul. 211, pp. 3-17, figs. 7), in a bulletin of the Wisconsin Experiment Station and some data given regarding its fat content, keeping qualities, etc.

An experimental study of the production of a dairy product called "whey butter" has been reported by C. F. Doane (U. S. Dept. Agr., Bur. Anim. Indus. Circ. 161, pp. 7).

G. A. Olsen (Jour. Biol. Chem., 5 (1908), No. 2–3, pp. 261–281) reports data regarding a proteid found in milk, cream, and but-

ter which he considers new. The chemical and physical character of this proteid are described.

The majority of investigations with fruits and nuts carried on in the United States have had to do with the methods of cultivation, transportation, and shipment rather than with composition, food value, and use in the home.

Cactus fruits, particularly tuna or the fruit of the prickly pear, which is used in southwestern United States and to a greater extent in Mexico as a foodstuff, were studied with respect to its composition and nutritive value, by R. F. Hare and D. Griffiths (New Mexico Sta. Bul. 64, pp. 88, pls. 7, figs. 2).

In the study of the tuna as food for man, by D. Griffiths and R. F. Hare (U. S. Dept. Agr., Bur. Plant Indus. Bul. 116, pp. 73, pls. 6), information is given regarding the use of the fruit for jelly making and preserves as well as for other purposes.

Italian lemons and their by-products and methods of producing lemon oil and citric acid commercially are discussed in a summary of data by E. M. Chace (U. S. Dept. Agr., Bur. Plant Indus. Bul. 160, pp. 35–50, pls. 3, figs. 2).

In connection with a summary of data on the dietetic value of fruit, W. R. Lazenby (Trans. Mass. Hort. Soc., 1910, pt. 1, pp. 89–97) reports data regarding the water content of well-developed and undeveloped specimens. Less than 80 per cent water was found in undeveloped strawberries, peaches, and apples, as compared with 90 per cent in fine but not overgrown specimens. It is further stated that 92 per cent of water was found in fine large peaches, in comparison with 84 per cent in small peaches of the same variety.

Data were also recorded regarding the percentage of shell or waste, and edible portion in nuts, and similar factors. According to the author, there is a loss of nearly 2 per cent of the total weight of kernels in milling or cracking some of the larger sorts of nuts.

Various topics concerned with the composition, nutritive value, and use of fruit as food have been discussed in a summary prepared by C. F. Langworthy (U. S. Dept. Agr., Farmers' Bul. 293, pp. 38, fig. 1).

The occurrence of sucrose in grapes was studied by W. B. Alwood and his associates (Jour. Indus. and Engin. Chem., 2

(1910), No. 11, pp. 481, 482) with a number of varieties. The quantities found in the juice of 3 well-known varieties ranged from 4.49 and 5.66 gm. per 100 cc. of juice. In the juice of a new seedling it was considerably larger.

In a later report, W. B. Alwood (U. S. Dept. Agr., Bur. Chem. Bul. 140, pp. 24) states that he and his co-workers have examined practically all the wine and table grapes grown in eastern United States, and with the exception of the varieties mentioned (Hayes, Pocklington, and Worden and a seedling), they did not find sucrose in appreciable quantities. Extended studies were also made of varieties grown in other regions and the variations in sugar and acid content studied during ripening. In Catawba grapes the sugar more than doubled after the berries began to color, while the acid was only about half as great. Similar data are reported for many other varieties.

W. P. Kelly (Jour. Indus. and Engin. Chem., 3 (1911), No. 6, pp. 403–405) has studied the composition of Hawaiian pineapples and found them to vary considerably, the sugar content ranging from 9.15 to 15.23 per cent, and the acidity from 0.22 to 1.16 per cent, and increasing generally as the sugar increased. On the whole, Hawaiian pineapples show much the same average composition as those grown elsewhere.

"Green pineapples contain less acidity that the ripe fruit and also a small percentage of fiber, reducing sugar, and sucrose. Dextrin and starch do not occur in important quantities in pineapples at any stage. The reducing sugars and sucrose stand in inverse ratio to that of the ripe fruit. In the ripening of pineapples gathered green, the most important chemical change that takes place is the conversion of reducing sugars into sucrose, but the total sugar content appears not to be increased. . . .

"During the normal ripening of the pineapple, a rapid accumulation of sugars and a slight increase in acidity take place. When the fruit becomes approximately half ripe, it contains at least three-fourths of its maximum sugars."

Bread, milk, vegetables, bananas, and rhubarb were included by H. Ackroyd, (Bio-Chem. Jour., 5 (1911), No. 8–9, pp. 400–406) in a study of the presence of allantoin in certain foods.

His general conclusions are that "the whole quantity of allan-

toin excreted by man on a milk and vegetable diet may be derived directly from the food. Milk, white bread, French beans, green peas, all contain small quantities of allantoin, while none could be isolated from eggs, bananas, or rhubarb."

The food value of nuts and the various ways in which they may be used in the diet have been discussed by M. E. Jaffa (U. S. Dept. Agr. Yearbook 1906, pp. 295–312, pl. 1, fig. 1; Farmers' Bul. 332, pp. 28, fig. 1), in a bulletin published in connection with the nutrition investigations of the Office of Experiment Stations.

In connection with a study of pecan culture, the marketing of pecans, and related questions by W. N. Hutt (Bul. N. C. Dept. Agr., 30 (1909), No. 9, pp. 50, figs. 25), the use of pecans as food is considered, and instructions given for cracking these nuts particularly for commercial purposes.

The care and marketing of vegetables have been more often studied than their composition and nutritive value.

Canned peas and beans of different grades were analyzed by W. L. Dubois (U. S. Dept. Agr., Bur. Chem. Circ. 54, pp. 9), in connection with commercial canning, and particularly with reference to the use of soaked peas and beans in place of the fresh vegetables. In general, the soaked peas had a higher water and starch content and a somewhat higher specific gravity than the fresh canned peas. The author is of the opinion that such determinations may prove useful in connection with physical examinations in judging of the character of such canned goods.

The crude fiber and the crude starch content of the soaked were higher than in the case of the fresh canned beans, though the differences were less pronounced when the results were reduced to a dry matter basis.

The recorded data furnished some information regarding the changes which take place during the growth and ripening of peas.

"As the pea matures the ash decreases, the starch increases, and the crude fiber decreases as a rule, while the conclusions to be drawn from the determinations of nitrogen and ether extract are less decisive. In the peas from one locality the amount of nitrogen decreases as the pea matured, whereas in the same vari-

ety from another locality this variation was not so apparent. Similar changes in composition appear in the canned vegetables. The analyses seem to indicate that during the process of canning the peas take up from 2 to 10 per cent of water. It is difficult from these results to draw any conclusions as to the changes taking place during processing. The principal value of the work . . . is to afford data for the comparison of commercial grades."

Marine algæ are important articles of diet of native Hawaiians. In connection with the work of the Hawaii Experiment Stations, Minnie Reed (Hawaii Sta. Rpt. 1906, pp. 61–88, pls. 4) studied the economic importance and food value of a large number of these marine algæ, reporting cooking tests in addition to analytical work and studies of the value of seaweed mucilage, gelatin, etc.

A digest of data on insoluble carbohydrates, particularly those of marine algæ, and a summary of digestion experiments carried on in the author's laboratory with such foods in comparison with raw Italian chestnuts, have been briefly reported by L. B. Mendel (Zentbl. Gesam. Physiol. u. Path. Stoffwechsels, n. ser., 3 (1908), No. 17, 641–654).

The character and nutritive value of carbohydrates of lichens, algæ, and related substances, particularly marine algæ, as studied by Mary D. Swartz (Proc. Amer. Soc. Biol. Chem., 1 (1910), No. 5, pp. 257, 258; Trans. Conn. Acad. Arts and Sci., 16 (1911), pp. 247–382), the hemicelluloses from 10 species of algæ were found to contain pentosans and galactans. The pentosans, with one exception, were largely found insoluble in cold water, while the gelactans were soluble and characterized by their gelatinous nature. Small quantities of soluble pentosans were associated with them in every case.

The resistance to bacterial action was studied, and digestibility was studied in vitro, and in other ways.

They were found to be very resistant to the action of animal and vegetable enzyms. Experiments showed that galactans were not affected by the ordinary aerobic bacteria of the alimentary tract, or by mixtures of soil and fecal aerobes, of soil and fecal anaerobes, or of powerful putrefactive organ-

isms such as *Bacillus anthracis symptomatici and B. maligni aedematis*. Pentosans, mannans, and levulans were found to be gradually decomposed by soil and fecal bacteria and by putrefactive anaerobes, sometimes with the formation of reducing substances.

"When introduced parenterally, either subcutaneously or intravenously, they are not retained or altered by the organisms, but are gradually excreted in the urine. Feeding experiments on dogs and human subjects show that those hemicelluloses most readily attached by bacteria disappear most completely from the alimentary tract. Galactans, which are unaffected to any appreciable extent, are excreted in amounts averaging 75 per cent; pentosans and mannans, hydrolyzed by bacteria, disappear almost entirely during the processes of digestion.

"The experiments give little justification for considering these carbohydrates as typical nutrients for man."

A popular digest of data regarding the composition, food value, digestibility, and place in the diet of potatoes and other root crops used as food is prepared by C. F. Langworthy (U. S. Dept. Agr., Farmers' Bul. 295, pp. 45, figs. 4).

Proprietary foods are made and marketed in large variety. Their composition and food value seem to have been studied much less frequently than many other commercial food products notwithstanding the fact that a knowledge of their real value would seem to be particularly important as they are chiefly recommended by the makers for use in infant feeding and in invalid dietetics.

The composition and true nutritive value of a number of proprietary foods and food products are discussed in a paper by Graham Lusk (Jour. Amer. Med. Assoc., 49 (1907), No. 3, pp. 201, 202, 270), dealing with the general subject of the nutritive value of such foods.

D. L. Edsall (Jour. Amer. Med. Assoc., 54 (1910), No. 3, pp. 193–196) also discusses this general question, as has J. Howland (Jour. Amer. Med. Assoc., 54 (1910), No. 3, pp. 196–201), who pays particular attention to predigested foods.

A published paper gives data regarding predigested foods and similar goods (Jour. Amer. Med. Assoc., 48 (1907), pp. 1612–

1614, 1694; 49 (1908), pp. 1294, 1295; abs. in Chem. Abs., 2 (1908), No. 12, pp. 1740, 1741).

G. F. Richmond and W. E. Musgrave (Philippine Jour. Sci., 3 (1909), No. 2, pp. 87–90) report a study of the composition of malted milk, particularly its fat content, which was found to be 8.18 per cent.

Experiment station investigators have given much attention to the breeding of cereal crops, the influence of fertilizers on composition, and other related questions. This work is perhaps more appropriately considered in connection with agricultural chemistry than with nutrition, though some of it, notably that with wheat and with corn, has an obvious relation to questions of human nutrition.

It is interesting to note that comparatively wide variations are observed in the composition of light and heavy kernels of wheat of the same variety, in the grain from well developed and imperfectly developed ears of corn, and in the kernels in different parts of the ear.

In a study of the improvement of corn, by A. M. Soule and P. O. Vanatter (Virginia Sta. Bul. 165, pp. 91–185, figs. 48), it was observed that many of the best yielding ears did not have as high a protein content as the undesirable ones.

C. L. Penny (Delaware Sta. Rpt. 1904–1906, pp. 13–33) found a wide range in protein content, the minimum being 6.25 in one crop and the maximum 12.69. The smaller kernels at the end of the ear were found to contain on an average 0.3 per cent less protein than the large and well formed kernels.

These matters have been extensively studied at the Illinois Experiment Station. In a report of investigations regarding ten generations of corn breeding, L. H. Smith (Illinois Sta. Bul. 128, pp. 457–575, figs. 2) summarizes data covering the range in protein and fat content. The results obtained show that starting with a single variety it was possible in ten generations to increase the protein content "from 10.92 per cent to 14.26 per cent, a gain of 3.34 per cent, while by breeding in the opposite direction it has been possible to reduce the protein content from 10.92 to 8.64 per cent, a reduction of 2.28 per cent, making a total difference between the two strains of 5.62 per cent. It is

further shown that the high-oil corn has increased from 4.70 per cent to 7.30 per cent of oil, while a low-oil corn has decreased from 4.70 per cent to 2.66 per cent, the difference between the two strains in 1906 being 4.71 per cent.

"High protein and low protein seed were planted together on one plat and high-oil and low-oil seed on another. These plats were continued for 3 years, and the results secured did not indicate that the soil influences the protein or the oil content.

"A study of the secondary effects produced by selection to change the composition of the grain indicated that the change in the composition of the grain has produced no very marked effect upon the composition of other parts of the corn plant."

The composition of corn and corn products, including green corn, their nutritive value and place in the diet, and similar questions have been discussed in a popular summary by C. D. Woods (U. S. Dept. Agr., Farmers' Bul. 298, pp. 40, figs. 2), published in connection with the nutrition investigations of the Office of Experiment Stations, which contains some individual work regarding the composition and digestibility of hulled corn and corn bread and some work regarding the composition of popcorn popped and unpopped.

Alice R. Thompson (Hawaii Sta. Rpt. 1908, pp. 51–58), of the Hawaii Experiment Station, has reported the results of studies of Japanese rice and Hawaiian-grown rice, both polished and unpolished, and rice paddy and straw from imported and Hawaiian rice and from rice grown under different conditions, the nitrogenous constituents being determined in every case, and proximate and ash analyses in the case of rice grain and rice straw and paddy.

Little variation was noted in the chemical composition of the different varieties of rice, and the author is of the opinion that the claim for superiority of Japanese imported over Hawaiian-grown rice is not substantiated so far as nutritive value is concerned.

Comparisons of the analyses of polished and unpolished grain showed that the unpolished rice contained about four times as much fat as the polished, as well as more protein, crude fiber, and ash. Practically all the nitrogen of the rice grain was found to be proteid nitrogen.

The question of the wholesomeness of polished and unpolished rice and the more specific question of the possible relation of polished rice to beri-beri are matters which have been given much experimental study in recent years as a part of the general question of the possible connection between the presence or absence of particular mineral constituents, protein radicals, or other constituents and the occurrence of the disease.

The matter of the possible relation of rice to beri-beri is of particular importance in the regions of the Orient where rice is the principal carbohydrate foodstuff, so naturally the question has been studied as a part of the scientific work undertaken by the Philippine Department of Science. H. Aron and F. Hocson (Biochem. Ztschr., 32 (1911), No. 3–4, pp. 189–203), in an investigation on rice as a foodstuff, have reported experimental studies in which the balance of income and outgo of nitrogen was determined on a rice diet supplemented by other foods chiefly of vegetable origin, including such material as bananas, rice polish, and phytin.

Analyses of a large number of samples showed that relatively more phosphorus than nitrogen was lost by polishing rice. The unpolished rice contained on an average from 0.7 to 0.8 per cent P^2O^5, undermilled rice from 0.4 to 0.6 per cent, and overmilled rice from 0.15 to 0.4 per cent.

The authors conclude that an exclusive rice diet will not supply protein enough to meet man's demands, and that therefore it must be supplemented by vegetable, or better, animal foods rich in protein. Such a mixed diet is satisfactory from an hygienic standpoint, provided the rice has not lost too much phosphorus by overmilling or polishing. From their experimental studies they conclude further that, for a man weighing 50 kg., a diet made up of rice supplemented by vegetable foods must contain at least 75 gm. protein in order to meet hygienic requirements, and that a diet of rice supplemented by fish or meat must contain at least 65 gm., of which at least $\frac{1}{3}$ is supplied by animal foods.

Information gained from practical experience with beri-beri and unpolished rice in the Philippines was summarized by V. G. Heiser (Philippine Jour. Sci., B. Med. Sci., 6 (1911), No. 3, pp.

229–233), particularly regarding the efforts which have been made to encourage the local use of unpolished rice and the success which has attended it.

For purposes of convenience, "a rice containing less than 0.4 per cent of phosphorus pentoxid is regarded as polished and that which contains a greater percentage of phosphorus pentoxid as unpolished rice."

An attempt to secure legislation regarding the use of unpolished rice in the Philippines is briefly discussed.

The question of cotton seed as human food has been considered by G. S. Fraps (Texas Sta. Bul. 128, pp. 5–15), who reports analyses of cotton-seed flour, cotton-seed flour bread, and other cotton-seed bakery products. The general conclusion is that cotton-seed flour is rich in protein and that it may be used alone or mixed with wheat flour for the preparation of appetizing foods. In the author's opinion, there is no reason to believe that cotton-seed flour will not prove a wholesome product when used in small amounts.

In his discussion, the author draws attention to the fact that cotton seed has more or less proved harmful when used as food for domestic animals, particularly pigs, but he is of the opinion that the quantities likely to be used would not prove harmful to man. Nevertheless, he cautions against using too large amounts.

It is interesting to note that F. Russell (Ann. Rpt. Bur. Amer. Ethnol., 26 (1904-5), pp. 66–92, figs. 7) states that cotton seed was formerly used as foodstuff by the Pima Indians of southern Arizona.

The widespread interest at the present time in the possibility of using cotton-seed meal as a food for man lends a special interest to the investigations which have been undertaken to determine the reason why it proves harmful to domestic animals, particularly pigs, when fed a considerable time in fairly generous quantities. Such studies will probably be referred to in detail elsewhere. It may be noted here that it seems to be the case that the renal disturbances or other pathological conditions observed when it is fed to pigs may be postponed or even in some cases materially lessened by feeding a large proportion of green fodder with the cotton seed.

Interesting investigations on the general question of the poisonous properties which cotton seed sometimes exhibits when fed to farm animals have been carried on in the Bureau of Animal Industry and reported by A. C. Crawford[a] (U. S. Dept. Agr., E. S. R., 22 (1910), No. 6, pp. 501–505). His conclusion is that the poisonous principle is not an alkaloid but probably an inorganic compound, namely, a salt of pyrophosphoric acid. Phosphoric acid has long been known to be present in cotton-seed meal in considerable quantity, and has been suggested as having a possible relation to its toxicity, but the methods of study followed have not been such as to bring out this relationship or lend support to the hypothesis.

The conclusions advanced are supported by a large amount of data from a systematic series of laboratory studies and physiological tests and have been further confirmed by feeding experiments with dogs carried on by the Bureau of Animal Industry which were not reported in the preliminary account of the work.

Not all cotton seeds exhibit poisonous properties, particularly being influenced in this respect by variety and by method of cultivation.

To quote from Dr. Crawford's conclusions, "the chief poisonous principle in certain cotton-seed meals is a salt of pyrophosphoric acid. In some, this salt seems to be a simple one, presumably inorganic, while in others, it is more complex, perhaps an organic one. Probably this difference in the combinations of pyrophosphoric acid may aid in explaining the variation in toxicity of different meals. In certain cotton-seed meals one would expect to find salts of metaphosphoric acid entering into this action. To be harmful, the pyrophosphates must be in such a form that they can be absorbed, or the phosphoric acid ionized in the gastro-intestinal tract. The harmlessness of certain cotton seeds and meal is mainly due to the fact that in them the phosphoric acid exists largely, if not entirely, as a compound of ortho, and not as one of the other phosphoric acids. Small amounts of pyrophosphates can apparently be borne without injury. The amount of the salt which may be permitted in cotton-seed meal should be determined."

([a] Jour. Pharmacol. and Expt. Ther., 1 (1910), No. 5, pp. 519–548).

Many summaries of data regarding the composition of foods have appeared, such work not infrequently forming a part of treatises on food and nutrition.

A set of fifteen colored food charts, prepared by C. F. Langworthy (U. S. Dept. Agr., Office Expt. Stas. Food and Diet Chart 15), has been issued in connection with the nutrition investigations of the Office of Experiment Stations, which are designed to show graphically the composition and energy value of the common food materials and to summarize some general data regarding the functions and uses of food.

Special Studies of Ash, Protein, and Other Food Constituents

No new products of particular importance have appeared during the period under consideration in this summary, though many of more or less general importance have been studied, including dairy products, fruits, meats, cereal grains and other materials. Methods of analysis as usual have received a great deal of attention.

Much work has been reported in connection with inspection of food under government and state pure food laws. No attempt can be made here to summarize this. As taken in connection with other pure food work, it constitutes a subject in itself.

In addition to studies of the composition of food already cited, a number of investigations have been reported which have to do with some detailed study of food constituents. For instance, the solubility relations of milk sugar, the vapor pressures of saturated solutions of hydrated milk sugar, the influence of concentration on the equilibrium between the forms of milk sugar, and other similar questions were studied by C. S. Hudson (Jour. Amer. Chem. Soc., 30 (1908), No. 11, pp. 1767-1783, figs. 2).

A bulletin by E. B. Forbes (Ohio Sta. Bul. 207, pp. 23–52), of the Ohio Station, on the balance between inorganic acids and bases in animal nutrition, endeavors to show the bearing on practical animal nutrition of the relationship between those mineral elements of our foodstuffs and of living animal tissues, which in the body give rise to inorganic acids, and the various

means at the disposal of the animal for accomplishing protection from these acids through effecting their neutralization. The relation of ash constituents to human nutrition in general is also considered. The investigations are reviewed in detail and a number of general deductions are drawn.

The available alkali in the ash of human and cow's milk in its relation to infant nutrition was studied by J. H. Kastle (Amer. Jour. Physiol., 22 (1908), No. 2, pp. 284–308). The salient points of difference between the ash of the two kinds of milk, the author points out, are: "Human milk contains relatively more of its mineral matter in utilizable form than cow's milk; it can supply the organism of the child with relatively larger amounts of available alkali in proportion to the proteid than cow's milk; it contains much less proteid; and it contains a more readily absorbable variety of fat."

The nature of the chemical combinations of potassium in the tissues was investigated by W. Koch and C. C. Todd (Abs. in Jour. Biol. Chem., 9 (1911), No. 2, pp. XV, XVI; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, pp. 9, 10). The results thus far obtained indicate that "sodium and potassium phosphatid compounds exist in all the tissues of the body and are probably of much more importance than the hitherto assumed ion-protein combination."

H. S. Grindley and E. L. Ross (Jour. Biol. Chem., 8 (1910), No. 6, pp. 483–493) have studied the determination of organic and inorganic phosphorus in meats. Judging from the data which they recorded, it appears that the coagulation of the protein of the aqueous extracts of flesh by heat does not change organic phosphorus to the inorganic form to any appreciable extent.

The subject has also been studied by P. F. Trowbridge and Louise M. Stanley (Jour. Indus. and Engin. Chem., 2 (1910), No. 5, pp. 212–215; abs. in Analyst, 35 (1910), No. 412, p. 311). The proportion of soluble organic phosphorus in total soluble phosphorus in meat was found to vary considerably in different animals and in different parts of the carcass of the same animal. The lowest recorded value (26 per cent) was observed with an emaciated steer, and the highest (91 per cent) with a fat show

steer. "During cooking, a progressive splitting up of the organic phosphorus compounds takes place, and in well-cooked meats practically the whole of the phosphorus is present in inorganic combination."

Data are presented by C. K. Francis and P. F. Trowbridge (Jour. Biol. Chem., 7 (1910), No. 6, pp. 481–501; 8 (1910), No. 1, pp. 81–93) regarding investigations of the kind and amount of phosphorus present in beef cattle in different conditions of fatness. The results were not uniform enough to warrant general deductions. No relation was evident between phosphorus and total ash.

The question was also studied with reference to the kind of phosphorus present in different cuts.

"The round cut of beef contains more phosphorus, in forms which are soluble in cold water than any of the other cuts. Phosphorus is found chiefly in the muscular or connective tissue; the fats contain but little. The flesh of a thin animal contains more soluble phosphorus than that of a fat animal. The quantity decreases with increasing fatness even when it is expressed on a moisture and fat-free basis."

The nature of the phosphorus compounds of the brain, both normal and diseased, was studied by W. Koch (Jour. Amer. Med. Assoc., 52 (1909), No. 18, pp. 1381–1383), the work in considerable part dealing with the phosphorus supply in the diet. The phosphorus required for the growth of the brain the author concludes is amply supplied by the phosphorus of our daily diet. "If desired, the addition of phosphorus-rich foods, such as eggs, sweetbreads (pancreas), liver, and some meats, can be made to meet further requirements, and will far exceed in amount the phosphorus obtained in less natural form from the prescribed doses of any of the various drugs in commercial use. The use of such foods is, however, limited by their richness and their tendency, on account of their rich fat content to interfere with gastric digestion.

"As far as the nervous system is concerned, the addition to the diet of commercial phosphorus compounds, such as hypophosphites, glycerophosphate, phytin, lecithin, etc., is to be discouraged because, in the first place, there is no conclusive

evidence that they have any effect on the growth of the brain, and, second, the amount usually recommended means only a very insignificant addition to the amount of phosphorus (even in its special forms such as lecithin) taken with the daily food."

The relation of brain phosphatids to tissue metabolites was studied by W. Koch and W. W. Williams (Jour. Pharmacol. and Expt. Ther., 2 (1910), No. 3, pp. 253–264). Some of the conclusions follow, which were drawn from experiments with substances which may be regarded as of food value to the tissues, including amino acids, glycocoll and glucose, and with substances having a characteristic physiological action, including among others adrenalin, caffein, and theobromin:

"The changes in state of aggregation of lecithin produced by sodium chlorid are the result of the independent action of the sodium and chlorin ions, whose effects are in opposite directions. Below the concentration of a physiological salt solution (0.12 molecular) the action of the chlorin ion, which decreases the state of aggregation of the lecithin, predominates. Above the concentration of a physiological salt solution, the action of the sodium ion, which tends to increase the state of aggregation of lecithin, comes more and more into prominence.

"It has been suggested that, when the phenomenon of chlorid retention occurs, some change has taken place in the state of aggregation of the cell lipoids which allows this action of the chlorin ion to predominate to a still greater extent.

"Ammonia and bile salts possess the power of altering the physical state of aggregation of lecithin to such an extent as to permit of the conclusion that they can be of functional significance in altering the permeability of cell membranes. . . .

"The ability of the tissue metabolites to combine with lecithin, as measured by the changes in the physical state of aggregation produced by their presence, is in some cases considerable, in other cases entirely lacking. Thus hypoxanthin, creatin, creatinin, adrenalin, and ammonia salts show evidence of combination. Inosit is doubtful and urea is negative.

"The amino acids show varying powers of combination. The dicarboxy-acids, like acids in general, tend to increase the state of aggregation of lecithin."

In connection with the nutrition investigations of the Department of Agriculture, H. C. Sherman (U. S. Dept. Agr., Office Expt. Stas. Bul. 185, pp. 80) studied iron in food and its functions in nutrition, and reported the results of three metabolism experiments in which the balance of income and outgo of nitrogen and iron and other mineral constituents was determined, as well as the results of two dietary studies undertaken with special reference to the iron content of the food consumed. Estimates were also made of the amounts of iron taken per man per day in 20 dietary studies made in connection with earlier nutrition investigations of the Office of Experiment Stations.

"Increase of iron," it is pointed out, "in the diet without a corresponding increase of protein is readily accomplished by the use of vegetable, fruits, and the coarser mill products of the cereal grains. In the experimental dietary here reported, the free use of such foods with milk but without meat or eggs resulted in an increase of 30 per cent in the iron content of the diet, while the protein, the fuel value, and the cost remained practically the same as in the ordinary mixed diet obtained under the same market conditions."

In continuation of the work on mineral constituents, H. C. Sherman, A. J. Mettler, and J. E. Sinclair (U. S. Dept. Agr., Office Expt. Stas. Bul. 227, pp. 70) have studied calcium, magnesium, and phosphorus in food and nutrition, reporting the results of 6 experiments on the metabolism of these constituents and a study of the amounts present in typical American dietaries. In general, the investigations show the importance in the diet of calcium, magnesium, and phosphorus and the possibility of securing them by the use in proper proportion of ordinary food materials.

To quote, "it is entirely feasible to increase largely the calcium and phosphorus intake by making a more liberal use of milk in the dietary. The same may, of course, be said of the various milk products in which the calcium and phosphorus compounds are largely or wholly retained, such, for example, as cheese, junket, kumiss, buttermilk, or cream. This is probably the simplest and more effective means of improving the dietary as regards calcium and phosphorus compounds, without decreasing

its acceptability or materially increasing its cost and with distinct advantages in other directions."

The balance of acid-forming and base-forming elements in foods, was studied by H. C. Sherman and J. E. Sinclair (Jour. Biol. Chem., 3 (1907), No. 4, pp. 307–309). Peas, milk, and prunes are the foods studied containing an excess of base-forming over acid-forming elements. With beef, oatmeal, and entire wheat grain the reverse was the case. It is obvious, the authors note, that "by the free use of meats and breadstuffs on the one hand or of fruits, vegetables, and milk on the other, the net excess of acid or base introduced into the body through the food may be varied at will within wide limits."

The very important work of T. B. Osborne and his associates on the cleavage products of protein has been continued, the studies reported having to do with the hydrolysis of excelsin (Amer. Jour. Physiol., 19 (1907), No. 1, pp. 53–60, pl. 1); hordein (Amer. Jour. Physiol,. 19 (1907), No. 1, pp. 117–124); legumin from the pea (Jour. Biol. Chem. 3 (1907), No. 3, pp. 219–225); glycinin from the soy bean (Amer. Jour. Physiol., 19 (1907), No. 4, pp. 468–474); the crystalline globulin of the squash seed (*Cucurbita maxima*) (Amer. Jour. Physiol., 19 (1907), No. 4, pp. 475–481); amandin from the almond (Amer. Jour. Physiol., 20 (1908), No. 4, pp. 470–476); the proteins of maize (*Zea mays*) (Amer. Jour. Physiol., 20 (1908), No. 4, pp. 477–493); gliadin from rye (Amer. Jour. Physiol., 20 (1908), No. 4, pp. 494–499); vicilin from the pea (Jour. Biol. Chem., 5 (1908), No. 2–3, pp. 187–195); legumelin from the pea (Jour. Biol. Chem., 5 (1908), No. 2–3, pp. 197–205); fish muscle (Amer. Jour. Physiol., 23 (1908) No. 2, pp. 81–89); vitellin from the hen's egg (Amer. Jour. Physiol, 24 (1909), No. 1, pp. 153–160); muscle of scallop (Amer. Jour. Physiol., 24 (1909), No. 1, pp. 161–169); crystalized albumen from hen's egg (Amer. Jour. Physiol., 24 (1909), No. 2, pp. 252–262); ox muscle (Amer. Jour. Physiol., 24 (1909) No. 5 pp. 437–446); casein (Jour. Biol. Chem., 9 (1911), No. 3–4, pp. 333–353); and wheat gliadin (Jour. Biol. Chem., 9 (1911), No. 5, pp. 425–438).

These very important investigations are too extended for summary here.

Supplementing his work on the cleavage products of protein T. B. Osborne and H. G. Wells (Jour. Infect. Diseases, 8 (1911), No. 1, pp. 66-124) have studied the biological reactions of the vegetable proteins, using the globulin from castor bean, flax seed, and squash seed, edestin from the hemp seed, excelsin from the Brazil nut, proteins from the cocoanut, legumin from the vetch, legumin and vicilin from the pea, vignin from the cowpea, glycinin from the soy bean, gliadin from wheat and rye flour, hordein from barley, and zein from maize. All of these were found to produce typical anaphylaxis in sensitized animals, the condition possessing all of the characteristics which are present when anaphylaxis is produced with serum or other animal substances containing soluble proteins.

It was found that considerable differences in toxicity were produced by the various proteins. "The most toxic proteins, as measured by the frequency of severe and fatal reactions, were the globulin of the squash seed, vignin, excelsin, and castor-bean globulin, which usually caused death when given in 0.1 gm. doses to properly sensitized animals. Edestin caused the least severe reactions of any of the proteins, while hordein and glycinin seldom caused fatal reactions; nevertheless, the minimum sensitizing and intoxicating doses of edestin and squashseed globulin are essentially the same."

The experiments showed, furthermore, that where continuous feeding was done with the proteins, the guinea pigs became immune to the proteins and could not be sensitized to them. There was a marked specificity shown within certain limits by the proteins, and a close similarity, if not identity, of the legumins of the pea and vetch and the close relation to the vicilin of the pea was shown by the interaction of these proteins. The probable identity of the gliadin from wheat and rye, or at least their near reaction, was also established. "In some instances doubtful results were obtained, for example, with some guinea pigs castor-bean globulin and flax-seed globulin interacted strongly, while with others similarly treated, no reactions were obtained."

The structure of proteids, enzyms and their relation to biological problems, and related questions are discussed in a paper by R. H. Chittenden (Science, n. ser., 27 (1908), No. 685, pp. 241–254).

D. D. Van Slyke and P. A. Levene (Proc. Soc. Expt. Biol. and Med., 6 (1908), No. 1, pp. 11-13) have reported studies of the cleavage products of plastein, the "protein-like substance or substances precipitated from concentrated albumose solutions by the action of enzyms." Their results "indicate that the plastein is related to the higher albumoses, and apparently, from the resistance of alkali, to the antialbumoses rather than to the native proteins."

T. B. Robertson (Jour. Phys. Chem., 13 (1909), No. 6, pp. 469–489) reported data which showed that the concentration of casein solutions can be very accurately studied by determining their refractive indices. His investigations are discussed at length. He later reported the results of studies of the refractive indices of solutions of certain proteins including the para-nucleins (Jour. Biol. Chem., 8 (1910), No. 4, pp. 287–295), serum globulin (Jour. Biol. Chem., 8 (1910), No. 6. pp. 441–448), casen in alcohol-water mixtures (Jour. Biol. Chem., 8 (1910), No. 6 pp. 507–511), and gliadin (Jour. Biol. Chem., 9 (1911), No. 3-4, pp. 181–184).

Cooking in its Relation to Nutritive Value.

The chemical changes involved in cooking processes have been investigated with a considerable number of materials.

Some data regarding army rations, field ranges, ovens, fireless cookers, the kitchen touring car, portable gas cooker for army use, etc., are included in a report of the U. S. Commissary General, H. G. Sharpe (Rpt. Commis. Gen. [U. S. Army], 1909, pp. 11–15).

Suggestions for a diet kitchen equipment, particularly with reference to naval hospitals, are presented in a paper by W. Wierzbicki (U. S. Naval Med. Bul., 4 (1910), No. 2, pp. 161–163, dgms. 2).

Studies of housekeeping efficiency as a private enterprise, by C. Barnard (Housekeeping Expt. Sta. [Conn.] Bul. 11, pp. 20, pls. 3) form the basis of a discussion of the increased efficiency through correct house planning, the use of conveniences and labor saving devices, the elimination of needless work, and similar questions. Some data are recorded regarding the labor involved in performing a definite household task by different methods.

Studies of the supposed connection between protein coagulation and the heat shortening of muscles were reported by E. B., Meigs (Amer. Jour. Physiol., 24 (1909), No. 1, pp. 178–186 dgms. 6), and are interesting not only from the standpoint of physiological chemistry but also because of their possible bearing on the changes which take place in animal foods during cooking processes. The facts reported, as the author points out, do not preclude "the possibility that the precipitation of protein from its solutions and the shrinkage of animal tissues under the influence of heat may be fundamentally more or less similar processes. They do show, however, that the shortening of striated muscle at temperatures above 50° is independent of the coagulation of myogen, and they make it seem probable that the heat shortening of most animal tissues is dependent, not on the aggregation of the particles of coagulable protein, but on some other process."

Elizabeth C. Sprague and H. S. Grindley (Univ. Ill., Univ. Studies, 2 (1907) No. 4, pp. 37, pls. 4, dgms. 10) studied the cooking of beef with a view to formulating methods which would give uniform results. In connection with this work, temperatures were recorded of the interior of the beef during cookery. If the temperature ranges from 55 to 65°C., the beef will be underdone or rare and red in color. At a temperature of 65 to 70°, it sill be medium underdone, and at a temperature of 70 to 80°, it will be well done.

In connection with his work showing the palatability and wholesomeness of spleens, and studies of their preparation for the table, E. T. Williams (Amer. Med., n. ser., 2 (1907), No. 9, pp. 522, 523) points out that although the raw spleens do not keep well, the cooked material, particularly boiled, has excellent keeping qualities. Attention is directed to the high iron and phosphoric acid content of spleens.

The economical use of meat in the home and many questions which have to do with the nutritive value of meat and the preparation of meat for the table, have been discussed in a popular summary by C. F. Langworthy and Caroline L. Hunt (U. S. Dept. Agr., Farmer's Bul. 391, pp. 43 II).

The question of cooking the cheaper cuts of meat is considered on the basis of the author's experimental study of the problem,

by C. Barnard (Housekeeping Expt. Sta. [Conn.] Bul. 6, pp. 17, dgm. 1).

The results of an extended series of artificial digestion experiments on starch of different sorts as affected by cooking were reported by Edna D. Day (U. S. Dept. Agr., Office Expt. Stas. Bul. 202, pp. 42, figs. 6) in a bulletin of the Office of Experiment Stations. Different sorts of ferments were used with potato, wheat, corn, and other starches. The conclusion was reached that potato, arrowroot, and probably tapioca and sago starches, are made not more easily digestible by long continued cooking, while the reverse is true with cereal starches, though the changes occur very slowly. In general, the experimental data reported are discussed with reference to household problems.

The effect of cooking on cellulose was studied by Edna D. Day (Jour. Home Econ., 1 (1909), No. 2, p. 177), who did not find that cell walls of potatoes when boiled or baked are ruptured, as is generally stated to be the case. When cells from cooked potatoes were examined, it was found that the middle lamella which holds the cells together had dissolved and that the cells had separated from each other, but the cell walls were not ruptured. "If, however, saliva is added to these unbroken cells, the starch filling them is very quickly digested, as shown by the fact that they no longer give the blue color with iodin, proving that the breaking of the cell wall is not at all essential for ease of digestion."

Studies of the etiology of pellagra reported by W. H. Buhlig (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 417–435, figs. 2), particularly with reference to the possible connection of Indian corn with this disease, did not lead to definite results. In connection with culture tests with moldy corn, some cooking tests were made, as certain molds are known to be resistant to heat, and the idea has been advanced that such enzyms may survive cooking. Corn meal mush and hominy made in the usual way, by boiling about 2 hours, was found to be sterile.

In other experiments carried on in the same public institution as Buhlig's work, the question was further studied by J. F. Siler and H. J. Nichols (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 437–478, figs. 8), who repeatedly found in corn meal and

hominy a spore-bearing bacterium which survived steaming for 2 hours.

A brief note on the effects of adding sugar to acid fruit at the beginning and end of the cooking period was published by Edna D. Day (Jour. Home Econ., 2 (1910), No. 1, p. 94), as the result of tests with cranberries, grapes, and apples, and also of the comparative sweetness of solutions of the same strength of cane sugar and a mixture of levulose and dextrose.

The conclusion is reached that "in cooking such fruits as apples, cranberries, and grapes, while the product is slightly less sweet if the sugar is added at the beginning than it is if it is added at the end, still the difference is too small to be of practical importance."

The problems of cookery at high altitudes (diminished air pressure) are discussed on the basis of experiments, by Mrs. A. Anderson (Boston Cooking-School Mag., 14 (1910), No. 8, pp. 372, 373, XVI, XVIII, XX). Data on this subject have been summarized in a recent paper (Jour. Home Econ., 3 (1911), No, 2, pp. 176–178).

According to experiments briefly reported by Olive G. Patterson and Clara C. Benson (Jour. Home Econ., 2 (1910), No. 6, pp. 656, 657) on the setting of gelatin, this material may be freed from its mineral matter and tyrosin-holding impurities without affecting the gelatinizing power of its solutions. Boiling for 1 hour did not prevent gelatinization though long-continued boiling diminished it. With respect to the effect of citric and acetic acids, it was found that 4 per cent gelatin solutions containing citric acid to a concentration of 1 per cent would gelatinize in the cold after 15 minutes' boiling, but that after 10 minutes' boiling of a 3 per cent solution with 0.5 per cent citric acid the gelatinizing power had considerably decreased.

The question of bread has received less attention from investigators than it did a few years ago.

The relation of yeast to flavor in bread has been studied experimentally by Ruth A. Wardall (Jour. Home Econ., 2 (1910), No. 1, pp. 75–91), who concludes that the flavor of bread can not be determined by yeast and possibly is not even affected by it.

Since for obvious reasons the time allotted for fermenting bread is short, she regards it as quite possible that an insufficient opportunity to develop flavor is given.

Some experiments were also made upon the effects of adding malt extract to bread dough.

The leavening agent in salt-rising bread was studied experimentally by Winona Woodward (Jour. Home Econ., 3 (1911), No. 1, pp. 100, 101), who isolated an organism which was not a yeast.

H. A. Kohman (Nat. Assoc. Master Bakers [Proc.], 13 (1910), pp. 29–37, fig. 1) studied salt-rising bread making, reaching the conclusion that the fermentation is due to a bacterium and not a yeast. The bacterium was isolated and studied in pure culture.

Among general discussions of bread may be mentioned a paper by M. E. Jaffa (Nat. Baker, 14 (1909), No. 166, pp. 52, 54), which devotes considerable attention to the use of raisins in bread making.

A large amount of data regarding the character and nutritive value of bread of different sorts and similar topics is included in a popular summary, entitled "Bread and Bread Making," by Helen W. Atwater (U. S. Dept. Agr., Farmer's Bul. 389, pp. 47, figs. 7).

A large amount of data has been reported regarding the principles and practice of ice cream making, by R. M. Washburn Vermont Sta. Bul. 155, pp. 92, dgm. 1). The work is based on an exhaustive study of the subject, particularly from a commercial standpoint.

The question of cooking naturally involves the relative value of different kinds of equipment and other similar topics.

Labor and money-saving appliances are discussed in a publication of the American School of Home Economics (Bul. Amer. School Home Econ., Ser. 1, 1908, No. 11, pp. 47, figs. 54), and numerous other discussions have appeared in journals and reports which have to do with such topics.

The matter of fireless cookers has been studied by a number of investigators.

Fireless cookers of special construction have been tested in connection with experiments on the preparation of food made

by the subsistence department of the U. S. Army. This work and data regarding foods supplied in the Philippines are reported by H. G. Sharpe (Rpt. Commis. Gen. [U. S. Army], 1907, pp. 10–14).

The value of different materials for the construction of fireless cookers and the effects of amounts and density of material upon the conservation of heat were studied experimentally by Ellen A. Huntington (Bul. Univ. Wis., No. 217, pp. 38, figs. 10), the article as a whole being an interesting contribution to the subject.

The construction and use of the fireless cooker from a practical standpoint have been studied by Caroline B. Lovewell, Frances D. Whittemore, and Hannah W. Lyon (Topeka, 1908, pp. 211, figs. 11).

A similar summary is also included in the volume dealing with fireless cookers prepared by Margaret J. Mitchell (New York, 1909, pp. XII + 315, figs. 18).

Canning, Preserving, Handling and Storage

The question of canning and preserving food, the changes brought about by cold storage, and related matters have been studied by a number of investigators.

An exhaustive summary of data regarding the question of storage of food products in the District of Columbia is contained in the U. S. House of Representatives report (Report of hearings on H. R. 16925, to regulate the storage of food products in the District of Columbia—Washington: U. S. House of Representatives Committee on District of Columbia, 1910, pts. 1–14, pp. 1–279).

The gases contained in swollen canned goods were studied experimentally by F. O. Tonney and J. B. Gooken (Amer. Food Jour., 3 (1908), No. 6, pp. 20–23, figs. 3). In general, the authors note that the presence of nitrogen indicates putrefaction and carbon dioxid, fermentation, the two processes being often found to be distinct from each other.

G .W. Shaw (California Sta. Circ. 33, pp. 4–8, figs. 3) compared cane sugar and beet sugar with a view to determining whether or not there is ground for the belief that beet sugar is inferior to

cane sugar for jelly making and preserving purposes. His conclusion was that such is not the case and that beet sugar is entirely satisfactory for such purposes.

Information regarding packing house methods, shipping, keeping quality, and similar subjects is included in a summary of the results of field investigations in pomology by G. H. Powell (U. S. Dept. Agr., Bur. Plant Indus. [Circ.], June 7, 1907, pp. 4).

In a volume by G. T. Hamel, entitled "Modern Practice of Canning Meats" (St. Louis, 1911, pp. 100, figs. 19, dgm. 1), the theories of canning are discussed, equipment described, and recipes and formulas given. The subject is considered from the standpoint of the small plant as well as from that of the large establishment.

The principles of canning are discussed and directions for canning a large number of fruits and vegetables and for pickling and preserving meats and fish are included in a bulletin by G. McCarthy (N. C. Dept. Agr., Biol. Div., 1907, pp. 37), designed for the use of housekeepers.

An extended summary of statistics regarding canning and preserving fruits and vegetables, fish, and oysters was presented by E. K. Ellsworth (Bur. of the Census [U. S.] Bul. 61, pp. 9–48), in a publication of the U. S. Census Bureau.

The culinary qualities of dehydrated eggs, fruits, vegetables, and milk were reported upon by H. A. Dent (Navy Dept., Bur. Supplies and Accts., Mem. Inform. Off. Pay Corps) [etc.], No. 85, pp. 626, 627), of the U. S. Navy Department.

The general question of canning vegetables in the home is discussed in a popular summary prepared by J. F. Breazeale (U. S. Dept. Agr., Farmers' Bul. 359, pp. 16, figs. 9), of the Bureau of Chemistry.

A. W. Bitting (U. S. Dept. Agr., Bur. Chem. Bul. 119, pp. 37, pls. 2, figs. 5) discusses the problem of catsup making in connection with an experimental study of the spoilage of tomato catsup.

The cause of cloudy liquor on peas was investigated by E. W. Duckwell (Canner and Dried Fruit Packer, 29 (1909), No. 1, pp. 34, 36), who reached the conclusion that it was caused by starch from the peas and overheating in canning.

A popular summary of data on canning peaches has been published by H. P. Gould and W. F. Fletcher (U. S. Dept. Agr., Farmers' Bul. 426, pp. 26, figs. 14), of the Bureau of Chemistry.

A study of the preparation of sugared and dried pineapple has been reported by H. C. Gore (U. S. Dept. Agr., Bur. Chem. Circ. 57, pp. 8, fig. 1).

An experimental study of packing prunes in cans, with satisfactory results, was also reported by G. W. Shaw (California Sta. Circ. 33, pp. 1–3).

Studies of jelly and jelly making have been reported by Nellie E. Goldthwaite (Jour. Indus. and Engin. Chem., 2 (1910), No. 11, pp. 457–462, fig. 1). A number of small fruits were used as well as orange juice, skins, and whole fruit.

According to the author's summary, "in what is usually a waste product (the white inner skins of oranges and lemons) we have an abundant source of pectin from which excellent jelly can be made if properly acidified. . . .

"It was noteworthy that the purest pectin yet prepared in this research was obtained from oranges and lemons. It was isolated . . . and was reprecipitated three times. By long manipulation of the precipitated pectin (supported on a very fine cloth suspended from the corners) the liquid was so completely worked out of the substance that a powdery white body, somewhat starch-like in appearance, was obtained. This was dried in a current of dry hydrogen over sulphuric acid.

"Ash determinations of orange pectin so obtained showed less than 0.5 per cent of ash—of lemon pectin about 3.5 per cent. . . . No melting point of this pectin could be obtained, but the substance, when out of contact with air, chars strongly at 170° C. It is hoped to continue this work on the isolation and examination of pure pectin."

A chemical-physical study of jelly making was carried on at the Florida Experiment Station, by J. Belling (Florida Sta. Rpt. 1908, pp. CV–CIX), the investigations having particularly to do with the influence of preliminary heating, final temperature, and the percentage of water, sugar, and acid upon appearance and quality of guava jelly.

"In boiling of guava jelly, some acid (the natural acid of the ripe fruit) is absolutely necessary to change much of the sucrose into invert sugar, and if this does not take place then the sucrose crystallizes out. Too much acid (and probably too prolonged boiling) seems to make the jelly sticky from the excess of invert sugar, and also to alter the pectin so that it will not gelatinize. . . .

"The depth of color seems to be increased by additional amount of acid, prolonged boiling, and higher temperature at which the boiling is stopped."

Experiments of great value with reference to the handling and marketing of fruits have been carried on at the Department of Agriculture and elsewhere.

Many questions which have to do with the preparation for shipment and marketing in fresh condition of fruits of different sorts have been studied in a paper by A. V. Stubenrauch (U. S. Dept. Agr. Yearbook 1909, pp. 365–374, pls. 3), of the Bureau of Plant Industry.

The method of pre-cooling fruit for shipment is discussed by G. D. Kellogg (Cal. Fruit Grower, 40 (1909), No. 1120, p. 1) in comparison with results obtained by ordinary methods of shipment.

The problem of time and temperature in cold storage, particularly with reference to the Kieffer pear, was studied by G. H. McKay (Proc. N. J. Hort. Soc., 32 (1907), pp. 127–135).

Experiments on the processing of persimmons to render them nonastringent, carried on by H. C. Gore (U. S. Dept. Agr., Bur. Chem. Bul. 141, pp. 31, pls. 3, figs. 5), though regarding as preliminary, tend to show that carbonic acid gas may be substituted for saké fumes for this purpose with Japanese persimmons, and that, combined with the use of dry starch to prevent cracking of the fruit during the processing, should lead to the perfection and use of this method for the production of nonastringent persimmons which may be pared and eaten like an apple.

A. E. Vinson (Plant World, 10 (1907), No. 11, pp. 259–262) has studied exhaustively the composition of dates with special reference to stages of ripening, the possibility of stimulating ripening, and related questions. As a part of his work, a special study of the endo- and ektoininvertase of the date is reported by A. E.

Vinson (Jour. Amer. Chem. Soc., 30 (1908), No. 6, pp. 1005–1020). The invertase of the date, he notes, remains "insoluble in all ordinary solvents throughout its green stages, but becomes readily soluble on ripening. The change in the behavior of the invertase towards solvents coincides very closely in point of time with the passage of the tannin into the insoluble form."

The influence of chemicals in stimulating the ripening of fruits has been studied further by Vinson (Science, n. ser., 30 (1909), No. 774, pp. 604, 605), who found that dates could be successfully ripened by exposing them to a vapor of acetic acid for 12 or 15 hours. "At the end of this time they have become transparent nearly to the seed and will then ripen naturally without further treatment. The process can be accelerated by exposing them to sunshine, or more rapidly by heating for some hours to 45°C. The process, it is anticipated, will permit the shipping of dates green and ripening them at their destination as bananas are now handled. . . .

"After moderate treatment with acetic acid, the tannin of the date has not yet become entirely insoluble but all astringency disappears in the next few hours. The intracellular invertase, however, passes into solution to quite an appreciable extent immediately after the treatment, and probably other intracellular or insoluble catalytic agents are released simultaneously."

The fresh ripe date is very soft and will not bear shipment. The author believes that by treatment with acetic acid vapor, it may be successfully ripened after shipment, a deduction of much commercial importance.

Continuing his study of the stimulation of premature ripening by chemical means, the author has studied the effect of many other substances beside acetic acid vapor on the ripening of dates and finds that a comparatively large proportion, including among other proprionic acids, ethyl chlorid, chloroform, gasoline, ether, acetone, and volatile oils. A. E. Vinson (Jour. Amer. Chem. Soc., 32 (1910), No. 2, pp. 208–212).

Studies of the artificial ripening of dates by the aid of chemical substances were later reported in greater detail by A. E. Vinson (Arizona Sta. Bul. 66, pp. 403–435), and of ripening by incubation by G. F. Freeman (Arizona Sta. Bul. 66, pp. 437–456).

In a study of methods of canning meat with reference to proper disposal of defective cans, C. N. McBryde (U. S. Dept. Agr., Bur. Anim. Indus. Rpt. 1907, pp. 279–296, fig. 1) draws a number of general conclusions regarding putrefactive and fermentative changes in the contents of cans, and similar matters.

Packing oysters with and without ice and similar questions were studied by the Indiana State Board of Health, (Mo. Bul. Ind. Bd. Health, 10 (1908), No. 11, pp. 134–136, fig. 1) with reference to the pecuniary loss to the consumer and the melting of ice in the oysters, analytical data being reported.

The possibility of preserving eggs with a number of substances, including sodium silicate of different grades, was studied by R. Berger (Jour. Indus. and Engin. Chem., 3 (1911), No. 7, pp. 493–495; Reprint, pp. 4; Pure Products, 7 (1911), No. 8, pp. 423–425), who reports data regarding the permeability of the shells of preserved eggs and their loss in weight when kept in the open air after preservation in comparison with unpreserved eggs.

Bacteriological studies which have to do with the infection and preservation of eggs were carried on by G. H. Lamson, Jr. (Connecticut Storrs Sta. Bul. 55, pp. 203–214, figs. 7) at the Connecticut Storrs Station, with reference to the cause of decomposition and sources of infection of eggs, the part played by temperature, and the precautions to be observed in preserving eggs.

The diastatic enzym of ripening meat was studied by A. W. Peters and H. A. Mattill (Jour. Biol. Chem., 6 (1909), No. 2, pp. XXIX, XXX). When muscle is autolyzed, the sugar becomes greater, they conclude, provided the meat is fresh and edible; otherwise, the amount diminishes.

The nature of so-called "black spots" on chilled beef was studied by E. Klein (Meat Trades' Jour., 30 (1909), Nos. 1113, p. 234; 1114, pp. 260, 261, figs. 2), who attributes much to the growth of a fungus (*Oïdium carnis*), which is described.

R. Hoagland (U. S. Dept. Agr., Bur. Anim. Indus. Rpt. 1908, pp. 301–314), of the Bureau of Animal Industry, has studied the action of saltpeter upon the color of salted meat. The red color of uncooked salted meat, to which saltpeter has been added

as a preservative agent, is due, he concludes, to the presence of NO hemoglobin which is formed by the action on hemoglobin of nitric acid due to the reduction of nitrites within the meat. The reduction of saltpeter to nitrites takes place in the meat equally well in either an acid, or an alkaline medium. Neither saltpeter nor nitrites exercise a color-preserving action in meat. The brown color observed when meat is cured with an excessive amount of saltpeter is due to the action of nitrites upon hemoglobin.

In connection with a study of deterioration and commercial preservation of flesh foods, W. D. Richardson and E. Scherubel (Jour. Amer. Chem. Soc., 30 (1908), No. 10, pp. 1515–1564) report the results of a study of experiments on frozen beef, from which the conclusion is drawn that no decomposition is shown in frozen beef stored for a long period, judging by the values obtained for ammonia nitrogen, and hence that no bacterial decomposition occurred in the stored meat. They conclude further that the stored meat did not differ in flavor from the fresh meat and that cold storage below −9°F. is an adequate and satisfactory method for the preservation of beef for long periods.

W. D. Richardson and E. F. Scherubel (Jour. Indus. and Engin. Chem., 1 (1909), No. 2, pp. 95–102) also reported an extended series of analyses of meat kept at room temperature with and without preservatives and of meat stored at 2 to 4°C., and in some cases afterward frozen and held at −9 to −12°C.

"While there are some contradictory figures in the analyses of the samples which were frozen after being stored at 2 to 4°C., from the results the conclusion may fairly be drawn that freezing of meats at −9 to −12°C. arrests bacterial decomposition, but can not in any degree restore the product to its original condition."

The results of an extended study of the effects of cold storage upon beef and poultry are reported by A. D. Emmett and H. S. Grindley (Jour. Indus. and Engin. Chem., 1 (1909), Nos. 7, pp. 413–436; 8, pp. 580–597), as part of an experimental study of the chemistry of flesh. The samples included uncooked refrigerated beef stored for 22 days and for 43 days and frozen drawn and undrawn fowl. Tests were also made of the relative losses in the

cooking of refrigerated beef held in cold storage for varying lengths of time, and the chemical changes resulting therefrom. The cooked meats from the sample stored 43 days were higher in their moisture content than the sample stored 6 days and were therefore juicier, higher in soluble and insoluble dry substance, in nitrogenous, nonnitrogenous and total organic extractives, in fat, in total ash, and in soluble inorganic, total soluble, and total phosphorus. "Further, the percentages of total nitrogen, insoluble and total protein were practically the same as were those for the samples from the 6-day storage meat. Therefore, the cooked meats from the 43-day samples, judging from the chemical composition, were at least as nutritous as were those from the samples stored for the shorter period of time."

The dietetic value of refrigerated foods as a whole is discussed by S. Rideal (Cold Storage and Ice Trade Jour., 36 (1908), No. 4, pp. 32, 33) on the basis of experimental data. The action of diastase, he concludes, is not entirely prevented by cold, but is very much retarded. The tenderness and maturing of refrigerated meat he attributes not only to the action of sarcolactic acid but also to the gradual and limited work of natural enzyms (pepsin and trypsin) which cause a certain amount of predigestion.

The changes which take place in chickens in cold storage have been exhaustively studied by Mary E. Pennington (U. S. Dept. Agr. Yearbook 1907, pp. 197–206, pls. 7), of the Bureau of Chemistry, which led to the general conclusion that both microscopic study and the taste of the cooked fowl confirmed the fact that microscopically visible degeneration does take place during long-continued storage.

The effects of different methods of handling and storing poultry, particularly with reference to the subject of drawing poultry, and the storage of poultry and eggs have been discussed by Mary E. Pennington (Ice and Refrig., 40 (1911), No. 2 pp. 59–62, charts 6; Nat. Provisioner, 42 (1910), Nos. 4, pp. 16, 23, 24; 5, pp. 23, 24; U. S. Dept. Agr., Bur. Chem. Circ. 64, pp. 42, figs. 9).

A chemical study of drawn and undrawn poultry kept in cold storage was also reported by W. F. Boos (Ann. Rpt. Bd. Health

Mass., 39 (1907), pp. 263-283), and a bacteriological examination of such poultry by H. R. Brown (Ann. Rpt. Bd. Health Mass., 39 (1907), pp. 285–336). According to Brown's conclusions, "decomposition depends largely upon the presence of moisture in the tissues, for moisture is absolutely essential to bacterial growth. In freshly killed birds, ordinarily or properly drawn, the surfaces quickly become dry. In cold storage birds, no matter how they are drawn, the tissues will be moist, because of the melting of the crystals of ice. If properly drawn, there would be but few bacteria present capable of causing decomposition."

Much attention has been devoted to the question of the relative wholesomeness of drawn versus undrawn poultry, by E. W. Burke (Amer. Food Jour., 3 (1908), No. 9, pp. 7–10).

A study of the effects of cold storage on eggs, quail, and chicken was reported by H. W. Wiley, et al. (U. S. Dept. Agr., Bur. Chem. Bul. 115, pp. 117, pls. 13), the general results being unfavorable to this process when long continued.

The effect of low temperatures on ground chicken meat was studied by H. W. Houghton (Jour. Indus. and Engin. Chem., 3 (1911), No. 7, pp. 497–505), in comparison with the original chicken meat. The chemical changes which apparently take place "are (1) slight variations in the case of moisture and other extract; (2) a small increase of ammonia, especially in the case of the light chicken meat; (3) a decided increase of water-soluble nitrogen, total solids, and organic extractives in the light chicken meat, with a slight decrease of the same constituents in the dark meat; (4) a decrease of coaguable nitrogen in both varieties of chicken meat during the first 30 days, followed by a rise which did not reach that of the fresh sample; (5) an increase of amino acids in both kinds of chicken meat, with an increase and decrease of the proteoses and peptones respectively in the light and dark chicken meat."

Chicken fat has been studied extensively at the Bureau of Chemistry. Mary E. Pennington and J. S. Hepburn (U. S. Dept. Agr., Bur. Chem. Circ. 75, pp. 11) report the occurrence of lipase in the crude fat of chickens, and find that it can resist freezing for as long a period as 89 months. (pp. 1–7).

The oxidation of chicken fat by means of hydrogen peroxid was studied by J. S. Hepburn (pp. 8–11), particularly with reference to the effect of prolonged freezing.

"The changes in the fat of chickens during prolonged freezing are similar to the changes called forth by oxidation of the fat with hydrogen peroxid. The Hehner number and the saponification number increase simultaneously, and aldehydes are formed. The increase in saponification number may, therefore, be ascribed to the formation of slightly lower homologues of the fatty acids of fresh chicken fat, while the increase in Hehner number is doubtless due to the formation of aldyhdes and ketones of high carbon content. These changes in the chicken fat in situ are probably produced by the action of enzyms."

The preparation of cod and other salt fish for the market was studied by A. W. Bitting (U. S. Dept. Agr., Bur. Chem. Bul. 133, pp. 63, pls. 6, figs. 4), of the Bureau of Chemistry, who also reports the results of a study of the cause of reddening in fish. This change was found to be due to a micro-organism, a remedy being extreme cleanliness.

The bulletin gives information regarding the composition of salt used in curing, losses in weight during curing, the amount of salt taken up by fish marketed in different forms, and variations in moisture and salt content due to season, style of packing, and other conditions.

L. W. Thomas (North Dakota Sta. Spec. Bul. 24, pp. 179-194, fig. 1) has reported a study of wrapped and unwrapped loaves, with reference particularly to moisture content and keeping quality. The general conclusions drawn are as a whole plainly in favor of wrapping bread, though, as the author points out, wrapping did not prevent loaves from becoming stale after 36 or 48 hours.

H. L. White continued this work by studying the moisture and acidity of samples of wrapped and unwrapped bread. According to his summary, bread made under cleanly conditions from good quality materials did not grow acid, when wrapped or unwrapped, even after 108 hours. Bread wrapped while warm and while hot showed a slight increase in acidity in the inside of the loaf.

C. A. A. Utt (Bul. Kans. Bd. Health, 7 (1911), No. 3, pp. 52–60) has also reported tests of the effect of wrapping bread upon its quality. In general, the unwrapped bread, when kept for 4 or 5 days, lost about twice as much moisture as the wrapped loaf, while the acidity remained practically the same. The wrapped bread was in edible condition for twice the ordinary period.

As pointed out by H. G. Bell (Amer. Miller, 37 (1909), No. 4, pp. 280, 281, fig. 1), in a paper on stored flour, fungi and bacteria are the chief destructive agencies. Flour of different grades was studied with reference to the presence of bacteria and as protection against the growth of these low forms of life, the author suggests storage in well-lighted rooms.

The changes in the weight of stored flour and butter were studied in detail by J. T. Willard (Bul. Kans. Bd. Health, 7 (1911) No. 1, pp. 9–14). The greatest loss in flours stored for a year was 0.79 lb. per sack of about 48 lbs. Loss of weight in butter was determined by the method of packing. Prints wrapped in parchment paper and placed in paraffin cartons, packed in cases, remained practically constant in weight. The loss in weight is chiefly due to loss of moisture by evaporation or in other ways.

Changes which take place in the composition of unground cereals during storage were studied by S. Leavitt and J. A. Le Clerc (Jour. Indus. and Engin. Chem., 1 (1909), No. 5, pp. 209–302). The investigations extended over 2 years. The results demonstrated that "there is more or less change in all cereals under the influence of aging. These changes seem to take place whether the cereal is stored in the whole grain or is ground to a fine powder before storage. In the latter case, however, the changes take place more rapidly. We notice that the principal products which seem most susceptible to change are first the sugars and then the 70 per cent alcohol-soluble proteins, the 5 per cent K_2SO_4-soluble protein and the water-soluble proteins coagulated by so-called Stutzer's reagent.

"Corn, barley, and oats are most subject to loss of sugar during aging. On the other hand, many samples of wheat show a slight loss the first year and then quite a rapid gain in the sugar content, in some cases a gain 24 per cent of the total sugar present being noted at the end of two years."

Dietary Studies and Dietetics

General dietary problems have been considered by many writers with a view to making the work of the laboratory available and useful to the housewife.

Methods of calculating the results of dietary studies and similar topics are discussed in a publication of the American School of Home Economics (Bul. Amer. School Home Econ., Ser. 1, 1909, No. 13, pl. 1, figs. 13), especially with reference to the use of the so-called 100-calorie-portion-method of calculating.

Emma S. Jacobs (Jour. Home Econ., 3 (1911), No. 2, pp. 162–168) in a discussion of family dietetics gives menus for what she believes accurately planned dietaries for families, and a table of weight and cost of protein and energy in different food materials designed to facilitate the computation of the nutritive value of such dietaries.

Food customs and diet in American homes have been discussed in a popular summary of data by C. F. Langworthy (U. S. Dept. Agr., Office Expt. Stas. Circ. 110, pp. 32), which proposes dietary standards for mineral constituents as well as for protein and energy, and discusses dietary standards as distinguished from physiological requirements.

Nellie M. Dickinson (Ill. Agr., 12 (1908), No. 5, pp. 142–145) gives data regarding the preparation of a day's ration designed to conform to dietary standards.

Information regarding dietary habits, food supply, and living conditions of native tribes often appears in descriptive articles, books of travel, reports of ethnological investigations, and other publications not directly concerned with nutrition, which is of importance in discussions of nutrition problems as well as of general interest. As illustrations of such work the following may be cited:

The food of natives of the upper Yukon has been described by F. Schmitter (Smithsn. Misc. Collect., 56, No. 4, pp. 6, 7). The diet of these natives consists of fish, game, and berries, supplemented at the present time by vegetables bought at local stores, though until recently they lived on animal food.

The Mackenzie River natives, it is pointed out, live almost exclusively on meat, and the author states that they are robust and healthy.

A study of the food supply of Pima Indians has been reported by F. Russell (Ann. Rpt. Bur. Amer. Ethnol., 26 (1904–5), pp. 66–92, figs. 7). These Indians subsist on a mixed diet in which vegetable food predominates, but it would seem probable that the proportion of meat used was greater in the past than at present.

Much information regarding the kind and amount of food used by native Indian tribes in the southwestern United States and northern Mexico is included in a physiological and medical study of the Indians by A. Hrdlicka (Smithsn. Inst., Bur. Amer. Ethnol. Bul. 34, pp. IX. + 460, pls. 28, figs. 2). Meat, corn, some vegetables, and other foods make up a simple mixed diet. Cooking processes are described as well as dietary habits and customs.

In a volume on "Mexico" (New York and London, 1909, pp. 213–218, pl. 1) C. R. Enock reports data regarding the Mexican peons, or country people. Corn meal, the native beans, fat, and meat when it can be obtained, are the principle articles of diet.

Of popular summaries which contain data of interest from the standpoint of diet may be mentioned a paper by C. W. Furlong (Harpers' Mo. Mag., 120 (1910), No. 716, pp. 217-229, pl. 1, figs. 7, map 1), which gives considerable information regarding the character of the diet of the natives of Tierra del Fuego, which consists almost entirely of the meat of wild animals, birds, the blubber from stranded whale, fish, and mussels.

In a later paper by Furlong (Harpers' Mo. Mag., 122 (1911), No. 732, pp. 813–827, pl. 1, figs. 9, maps 2) additional information is given on the subject, particularly regarding the food customs and living conditions of the Tehuelches of the Patagonian pampas. Apparently, these natives live very largely upon the meat of mares and game.

Less work pertaining to the food consumption of families and groups has been reported than in some other branches of dietetics.

From data regarding the food of a poor family in Buffalo and one in Boston published by Emma O. Lundberg (Survey 23, (1910), No. 20, pp. 728–730) the protein and energy in the daily food have been calculated.

As part of its nutrition investigations the Office of Experiment Stations has reported the results of 4 dietary studies of farmers' families in Vermont (J. L. Hills), 70 in mountain regions in Tennessee (C. E. Wait), and 14 in Georgia (H. C. White). The cost of nutrients and energy, peculiarities of the diet, adequacy of the food supply, and similar questions are discussed, the results being compared with earlier work of a similar nature. As a whole the bulletin supplies a large amount of statistical and other data regarding living conditions in rural regions, particularly those remote from large centers of population where conditions are very different from those which prevail in towns, cities, and farms which are otherwise situated. (U. S. Dept. Agr., Office Expt. Stas. Bul. 221, pp. 142, pls. 4).

The report of E. T. Wilson (Ann. Rpt. Isthmian Canal Com., 1910, pp. 323–325), the subsistence officer in charge of the subsistence department, Isthmian Canal Commission, contains data regarding the kind and amount of food served to laborers in the Panama Canal Zone. Using the average values for the composition of foods as purchased, it has been calculated that the European laborers' messes would supply 201 gm. protein and 5,428 calories energy per person per day, and the common laborers' kitchens 148 gm. protein and 4,680 calories energy. The amounts actually eaten were not calculated, as no data regarding the waste and refuse were available.

The scientific work organized under government auspices in the Philippines has provided an opportunity for important studies of the native dietary. E. D. Merrill (Philippine Jour. Sci., B. Med. Sci., 4 (1909), No. 4, pp. 219–223) has studied the principal foods used by the natives of Taytay, and H. Aron (Philippine Jour. Sci., B. Med. Sci., 4 (1909), No. 4, pp. 225–231) has studied their food from a physiological standpoint. Rice is the staple nonnitrogenous food and is supplemented by fish, fruits, and some similar foods. The composition of a number of sorts of food was determined.

The matter has also been studied by V. G. Heiser (Ann. Rpt. Bur. Health Philippine Islands, 1909, pp. 25–29), his paper being entitled "Diet and Nutrition of the Filipino People."

That the diet in public institutions may now be passed upon by an expert in a manner profitable to the institution as well as of interest to the investigator is one of the important results of the nutrition work of the last 25 years or more. This has been obtained very largely as a result of the numerous investigations of dietaries in general and public institutions dietaries in particular, carried on as a part of the nutrition investigations of the Office of Experiment Stations and related enterprises.

A report of work of this character issued recently by the Department of Agriculture contains the results of studies in a home for old ladies and an orphan asylum in Philadelphia (Emma Smedley and R. D. Milner) and in orphan asylums, homes for the aged, and a public home whose inmates are chiefly middle-aged or aged people, in Baltimore (H. L. Knight, H. A. Pratt, and C. F. Langworthy). On the basis of the data reported the dietaries are critically considered and some changes suggested. The general problem of the dietary of children and the dietary of aged persons is discussed at length particularly with reference to public institutions. (U. S. Dept. Agr., Office Expt. Stas. Bul. 223, pp. 98).

Considerable information is given regarding the character of the diet in a state hospital for the insane in Illinois in connection with a study of the occurrence of pellagra at the institution, carried on by J. F. Siler and H. J. Nichols (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 437–478, figs. 8). On an average the simple mixed diet supplied approximately 30 gm. of protein and 2,000 to 2,500 calories per day.

In connection with an exhaustive and important investigation of the methods of fiscal control of state institutions carried on for the Sage Foundation, H. C. Wright (State Charities Aid Assoc. [N. Y.] Pub. 122, 1911, pp. 353) reports the calculated food supplied per man per year and per man per day in institutions in New York, Indiana, and Iowa, including hospitals for the insane, soldiers' homes, industrial schools, reformatories,

prisons, and institutions for the feeble-minded and for epileptics. The results are summarized as follows:

AVERAGE FOOD PER MAN PER YEAR AND NUTRITIVE VALUE OF DAILY RATION IN PUBLIC INSTITUTIONS

Location of institutions	Food per man per year		Food per man per day	
	Total amount	Cost	Protein	Energy
	Pounds		*Grams*	*Calories*
New York	1,227	$45.05	104.62	3,313
Indiana	1,176	43.03	98.78	3,429
Iowa	1,423	55.48	106.47	3,691

Numerous investigations of the food and diet of children have been made during the period under consideration. Though all that pertains to food from infancy to maturity is obviously of interest and value, special studies of infant feeding have not been included in this summary, since they are commonly regarded as pertaining to the subject of medicine rather than to dietetics.

The bearing of food during early life upon the normal development of the body is a question which has received attention from a number of investigators, the problem having been studied with young animals as well as with children.

In an investigation of this sort experimental studies with dogs were reported by H. Aron, together with the results of general observations on nursing children (Philippine Jour. Sci., B. Med. Sci., 6 (1911), No. 1, pp. 1-52, pls. 4, dgms. 5).

He concludes that "a growing animal which receives only sufficient food to keep its body weight constant, or to allow slight increase, is in a condition of severe starvation. If by a restriction of food the increase in weight is inhibited, the skeleton grows at the expense of other parts of the body, expecially of the flesh. Most of the organs retain their weight and size, while the brain grows to reach its normal weight. The composition of the body—when at a constant weight—undergoes remarkable

changes. Fat is consumed more or less entirely, the quantity of protein, especially of the muscles but not of the organs, is diminished and a great proportion of the body tissues is replaced by water; thus, this water and the increase of the skeleton together replace the body materials lost. The caloric value of 1 gm. body weight of an animal which has undergone such a process to its extreme limit may amount to only one-third of the normal value.

"It is possible by supplying suitable amounts of food to maintain a dog in an emaciated condition, apparently in good health, and at the weight of a puppy, for nearly 1 year, while its weight at the end of the year should be 3 times as great. If such an animal is thereupon fed amply, it fattens and rounds out, but does not reach the size of a control animal which from the beginning has been normally fed. It is unable to make good the growth suspended by the long restriction of food.

"The 'growth' principally depends on the tendency to grow possessed by the skeleton. The skeleton loses its capability of growing in more advanced age regardless of the size which the animal has reached."

A paper of importance in considering the question of physiological requirement and dietary standard has been published by H. J. Waters (Proc. Soc. Prom. Agr. Sci., 30 (1909), pp. 70–98; Separate, pp. 29, figs. 6), who discusses the influence of nutrition upon the animal form, as the result of a large number of experiments made with farm animals (beef cattle). In general, the results clearly show that a decreased supply of nourishment in the young animals hinders body development. The matter is also taken up in a later paper (Quart. Rpt. Kans. Bd. Agr., 29 (1910), No. 113, pp. 59–86, figs. 7).

The relationship of food to physical development is discussed by D. McCay (Philippine Jour. Sci., B. Med. Sci., 5 (1910), No. 2, pp. 163–170), on the basis of his investigations, his conclusion being that there is a close relationship between the nutritive value of the diet, and particularly its protein content, and physical development. This he believes is clearly brought out in a comparison of the degree of nitrogenous interchanges of a number of native races, arranged according to the amount of nitro-

gen per kilogram of body weight. At the head of the list as regards physical development are the Nepalese Bhutias, with 0.42 gm., and the Tibetan and Bhotan Bhutias, with 0.35 gm., respectively, of nitrogen per kilogram of body weight and with a large amount of animal food in the diet, and at the bottom of the list are the Bengalis and Ooriyas, with 0.116 gm. of nitrogen per kilogram of body weight.

Few dietary studies with children have been reported in the United States during the period under consideration.

The general question of the feeding of young children has been discussed in a popular way by Mary Swartz Rose (Teachers Col. [N. Y.] Bul., 2. ser., 1911, No. 10, pp. 10) and by others.

The dietary studies made in orphan asylums by the Department of Agriculture (U. S. Dept. Agr., Office Expt. Stas. Bul. 223) have been referred to elsewhere. The report of this work contains a discussion of children's dietaries and proposed dietary standards.

An extended summary of data regarding the daily meals of school children was prepared by Caroline L. Hunt (Bur. of Ed. [U. S.] Bul. 3, 1909, pp. 62, pls. 3, dgm. 1) for the U. S. Bureau of Education. The paper is an important contribution to the subject, not only from the material it brings together, but also because of the suggestions it makes regarding the rational feeding of school children.

School luncheons have also been studied under the auspices of the Women's Educational and Industrial Union (Ann. Rpt. Women's Ed. and Indus. Union, 29 (1908), pp. 34, 35).

Lillian D. Wahl (Charities and Commons, 20 (1908), No. 11, pp. 371–374) has reported data on this subject, particularly regarding attempts made to supply food to children in some of the New York public schools.

Contributions to the school luncheon problem are also made by Marion Bell (Boston Cooking-School Mag., 12 (1908), No. 6, pp. 292, 293) in an article describing a luncheon cooked and served in the Honolulu Normal School.

An important contribution to the general question is the study of malnutrition of children in New York public schools made by E. M. Sill (Jour. Amer. Med. Assoc., 52 (1909), No. 25, pp. 1981–

1985). He found that 83 per cent of the 210 cases observed depended practically for their diet on bread with tea or coffee. The importance of a highly nutritious dietary, with a large amount of protein, is recognized, and suggestions given regarding the preparation of such a diet.

Continuing his work regarding the diet of undernourished school children in New York City (Jour. Amer. Med. Assoc., 55 (1910), No. 22, pp. 1886–1891), Sill studied the dietary of 28 families with malnourished children in the thickly congested districts of New York City and of 6 families in more comfortable circumstances but where the children were also undernourished.

In the first group the food cost 19 cents per man per day, and supplied 95 gm. protein, 68 gm. fat, and 407 gm. carbohydrates, the fuel value being 2,614 calories. The families were engaged in active or moderately active muscular work.

In the fairly well-to-do families the diet cost on an average 35 cts. per person per day, and supplied 149 gm. protein, 115 gm. fat, and 569 gm. carbohydrates, the fuel value being 3,884 calories. The families were engaged in moderately active work.

Where the dietaries were up to or above the standard the malnutrition was attributed to such factors as close quarters, over crowding, eating candy between meals, tuberculous infection, enlarged tonsils, or other similar cause. The author states that in his experience such children contract disease much more easily and have less resistance than well-nourished children.

Information regarding undernourished children in New York City was collected by Frances Perkins (Survey, 25 (1910), No. 1, pp. 68–72). According to her summary, "physical disabilities of one kind or another are closely associated with malnutrition, and make it doubly dangerous."

W. C. Hollopeter (Jour. Amer. Med. Assoc., 53 (1909), No. 21, pp. 1727–1730), who has studied the character of breakfasts of over 2,000 school children, has also contributed important information concerning existing conditions with reference to children's diet.

Numerous plans for providing proper food for school children have been proposed, particularly with reference to needy or undernourished children.

A luncheon project which has proved successful in Philadelphia is described by H. H. Bonnell (Starr Center Assoc. [Rpt.] 1909, pp. 18–20, fig. 1), the different articles being sold for a penny.

The subject of penny luncheons for school children in the thickly congested districts of Philadelphia is discussed further by Alice C. Boughton (Psych. Clin., 3 (1910), No. 8, pp. 228–231, fig. 1).

Data regarding the serving of penny lunches to school children in Boston are reported by Ellen H. Richards (Jour. Home Econ., 2 (1910), No. 6, pp. 648–653).

A school luncheon costing one cent per person, which furnishes, in round numbers, 9 gm. of protein, is described by A. L. Benedict (Dietet. and Hyg. Gaz., 23 (1907), No. 7, p. 404).

Some work has been done with older students and pupils.

Daily menus for the school year are presented, together with the results of a dietary study for 1 month, in a report issued by the Institute for Colored Youth (Teachers' Training School) (Cheyney, Pa., 1909, pp. 48). The work was done as a part of a project to prepare at reasonable cost a rational diet with protein and energy in accordance with commonly accepted dietary standards.

Agnes Hunt (Ill. Agr., 12 (1908), No. 5, pp. 146–148) reports data regarding the nutritive value and cost of food served in a students' boarding-house.

From data reported by P. R. Kellar (Cooking Club Mag., 12 (1910), No. 11, pp. 10, 11), regarding the diet in a students' boarding house in the University of Minnesota, the daily food which cost 22 cts. per man per day was calculated to supply 105 gm. protein and 3,715 calories of energy.

Of special investigations of dietary problems the following may be cited.

The possible relation of diet to fatigue, particularly a diet containing the usual average amount of protein, is discussed by I. Fisher (Bul. Com. One Hundred Nat. Health [Washington], 1909, No. 30, pp. VIII+138) in a report on national vitality, its wastes, and conservation. The author is of the opinion, from experiments which he has made, that there is a

relationship between protein consumption and the occurrence of fatigue.

Studies carried on by P. A. Shaffer (Amer. Jour. Physiol., 22 (1908), No. 4, pp. 445–455) with healthy men support the belief that with sufficient food either an increase or a decrease of muscular activity within physiological limits has per se no effect upon protein metabolism, as indicated by the nitrogen and sulphur partitions in the urine. The investigation as a whole was undertaken to secure data regarding diminished muscular activity and protein requirement.

The effect of an ash-free diet was studied experimentally by H. W. Goodall and E. P. Joslin (Trans. Assoc. Amer. Physicians, 23 (1908), pp. 92–106) with healthy individuals, for experimental periods of 13 and 9 days, respectively. The results obtained do not indicate any marked changes in metabolism ascribable to the ash-free diet.

A contest entered into for a wager, in which 48 men endeavored to carry on the back a weight of 100 lbs., for approximately 10 miles, furnished results which were discussed with reference to the strength and endurance of the men by C. F. Langworthy (Science, n. ser., 33 (1911), No. 853, pp. 708–711). Of the number who entered the contest 6 completed the task, while the others dropped out at various stages. The information collected from a number of the men showed that they lived on a simple mixed diet. The energy expended in moving the body and carrying the load over the course was calculated to be 1,137 calories on an average for the 6 successful contestants, of which amount 707 calories would represent the energy expenditure for motion of forward progression and 430 calories for energy expended in moving the load.

Similar calculations for individuals and for groups are reported.

It seems fair to conclude that the men who engaged in the contest were, as regards their food, their occupation, and their general living conditions, representative of a very large group of our population who are living comfortably and meeting their daily obligations in a creditable manner, who are, in fact, living the average life of the average man, with its varied activities and interests.

In so far as the recorded data throw light on the subject, they indicate that the average man living the average life is capable of meeting body demands of considerable severity—a conclusion which perhaps few would question, but which it is interesting to consider in the light of numerical data.

DIGESTION

Studies of thoroughness of digestion have been reported as has work on various details of the general question. The tendency seems to be toward the investigation of special topics rather than the digestion as a whole. Experiments have also been made with reference to ease of digestion, which involve the use of the respiration calorimeter. (See p. 73).

Colloid-chemical aspects of digestion are considered by J. Alexander (Jour. Amer. Chem. Soc., 32 (1910), No. 5, pp. 680–687), who reports ultramicroscopic observations. The author's general conclusion is that chemical analysis alone is not sufficient to express the digestibility and availability of food and that our consideration of such problems must be broadened.

The absorption of fat was studied by L. B. Mendel (Amer. Jour. Physiol., 24 (1909), No. 5, pp. 493–496) who used samples stained with Sudan III. He concludes that "when fat stained with water-soluble dyes, like Sudan III, is fed, the pigments readily pass into the lymphatic vessels and thereby reach the blood stream. Since these compounds are soluble in free fatty acids as well as in neutral fats, their presence in the lymph cannot be taken as evidence either for or against the possibility of the digestion of fats prior to their absorption."

The effect of the presence of carbohydrates upon the artificial digestion of casein was studied by Nellie E. Goldthwaite (Jour. Biol. Chem., 7 (1910), No. 2, pp. 69–81), the recorded data showing, according to the author, that each of the carbohydrates tested (glucose, maltose, dextrose, dextrin, and galactose) retarded the digestion of casein, the retardation being proportional to the amount of added carbohydrate.

The results of experiments by C. H. Neilson and D. H. Lewis (Jour. Biol. Chem., 4 (1908), No. 6, pp. 501–506, fig. 1) on the

effect of diet on the amylolytic power of saliva led them to conclude that "there is a change either in the amount of ptyalin or in its activity, or in the concentration of the saliva, which enables more or less starch to be digested with a given quantity of saliva according to the diet. . . . Whether this change in the amylolytic power of the saliva due to diet should really be called an adaptation to diet is immaterial."

G. Lusk (Amer. Jour. Physiol., 27 (1911), No. 5, pp. 467, 468) has summarized data accumulated in connection with some of his earlier experiments, which have to do with the questions as to whether dextrose arises from cellulose in digestion, the conclusion being that such is not the case.

According to the conclusions reached by J. H. Pratt and L. H. Spooner (Trans. Assoc. Amer. Physicians, 25 (1910), pp. 614–635) in a study of the internal functions of the pancreas in carbohydrate metabolism, there is a rapid decrease in the power to assimilate glucose after the onset of atrophy of the pancreas.

Using dogs with Pawlow fistules, N. B. Foster and A. V. S. Lambert (Proc. Soc. Expt. Biol. and Med., 4 (1906), No. 1, p. 13) studied the influence of water on gastric secretion and the chemical affinity of mucus for hydrochloric acid in the stomach.

"It was observed that with definite amounts of cracker meal as food, the amount and rate of gastric secretion depend to some extent on the amount of water given the dog with his meal, i.e., when small amounts of water are given, the secretion is slow and scanty. If larger quantities of water are mixed in the food the secretion is more abundant.

"The degree of acidity of gastric juice depends upon the amount of secretion. When this is considerable it is much more acid than when the secretion is scanty. . . . The proportion of free acid depends upon the amount of mucus secreted, since mucus protein like other proteins combines with HCl. Mucus in the presence of pepsin combines with HCl to a considerable extent and undergoes digestion, with formation of proteoses."

The results of a large number of experiments carried on by the Office of Experiment Stations coöperating with the Bureau of Animal Industry on the digestibility of cheese of different

sorts have been reported in a summary prepared by C. F. Doane (U. S. Dept. Agr., Bur. Anim. Indus. Circ. 166, pp. 22). In the first series there were 184 tests with 65 young men serving as subjects, and in the second series about 50 experiments. American cheese made by the regular Cheddar process, with varying amounts of rennet and cured for different lengths of time and ripened under controlled conditions, was used in the tests, as well as a number of other sorts of cheese, with a view to determining whether thoroughness of digestion was influenced by the kind of cheese, by the degree of ripeness, and by similar factors.

In general, cheese of all sorts was found to be very thoroughly digested and little or no difference in the comparative digestibility of cheese at different stages of ripeness was observed. It was also found that different kinds of cheese closely resembled cheese made by the Cheddar process in thoroughness of digestion, and, in general, "that all kinds of cheese, even the very high-flavored and so-called condimental cheeses, have a high food value."

Brief statements are also made regarding the experiments on the ease of digestibility of cheese in which the respiration calorimeter was used.

The digestibility of white of egg as influenced by the temperature at which it is coagulated was studied by P. Frank (Jour. Biol. Chem., 9 (1911), No. 6, pp. 463–470, dgms.2). The progress of the hydrochloric acid action and the total digestion is most rapid in the albumin not heated beyond 75°C.

The results are reported of 66 natural and 99 artificial digestion experiments with meat undertaken by H. S. Grindley, T. Mojonnier, and H. C. Porter (U. S. Dept. Agr., Office Expt. Stas. Bul. 193, pp. 100), to determine the ease and thoroughness of digestion of different kinds and cuts of meat cooked in a variety of ways. As regards thoroughness of digestion, the results do not indicate that very appreciable differences exist, and meats of all kinds and cuts can be classed with the very digestible foods, about 98 per cent of the protein and fat being retained in the body on an average.

The erepsin of cabbage has been studied by Alice F. Blood (Jour. Biol. Chem., 8 (1910), No. 3, pp. 215–225). It splits

tryptophan from Witte's peptone and casein, and tyrosin from peptone "Roche." It clots milk and liquefies gelatin. It does not digest fibrin, coagulated egg white, or edestin in neutral, acid, or alkaline solution, or in the presence of HCN. Other characteristics are given.

An extended study of the digestibility and nutritive value of legumes was carried on by C. E. Wait (U. S. Dept. Agr., Office Expt. Stas. Bul. 187, pp. 55), of the University of Tennessee, in connection with the nutrition investigations of the Office of Experiment Stations. In general, kidney beans, white beans, and cowpeas of different sorts were found to supply from 70 to 83 per cent digestible protein and from 87 to 96 per cent digestible carbohydrates. When the digestibility of a diet containing a considerable quantity of beans was considered, rather than that of the beans alone, the values were higher. The experiments as a whole demonstrate the great nutritive value of cowpeas.

A number of foods, particularly tropical fruits and vegetables, were analyzed by L. H. Merrill (Maine Sta. Bul. 158, pp. 219–238), of the Maine Experiment Station, and digestion experiments with hulled corn reported. These show in general that the digestibility of protein and the availability of energy are low in comparison with results obtained with white bread. Analysis of corn before and after popping showed that except for loss of water little change in composition was produced by this process.

Studies of digestibility of carbohydrates of marine algæ by Mary D. Swartz have been referred to elsewhere.

Metabolism; Respiration Calorimeters, Bomb Calorimeters, and Experiments with Them

A fairly large proportion of the work reported during the period under consideration has had to do with the metabolism of nitrogen and other constituents, including numerous studies of the metabolism of the income and outgo of energy made with the respiration calorimeter, and with special studies of body excretions, the influence of different food constituents

upon protein consumption, the effects of water drinking, and similar topics.

The average daily excretion of uric acid of 10 men in normal condition, ranging from 19 to 29 years, and fed a normal diet, was determined by P. J. Hanzlik and P. B. Hawk (Proc. Soc. Expt. Biol. and Med. 6 (1908), No. 1, pp. 18, 19, and found to be 0.597 gm., a value somewhat lower than the generally accepted average of 0.7 gm.

"The average daily protein ingestion from these same subjects, when permitted to select their diet, was 91.2 gm. or 1.33 gm. for such a period."

The metabolism of some purin compounds in the rabbit, dog, pig, and man was studied by L. B. Mendel and J. F. Lyman (Jour. Biol. Chem., 8 (1910), No. 2, pp. 115–143).

In the experiments with man hypoxanthin nitrate, xanthin, guanin, and adenin were added on different days to a purin-free diet. According to the authors, the examination of the urine showed that all four purins produced a marked rise in urinary acid and a small, yet noticeable increase in the elimination of purin bases. The smaller and lighter of the two subjects excreted, in every case, a larger percentage of uric acid and purin bases than the other subject, and, according to the authors, "may possess a more limited power for uric acid destruction."

The effect of meat purins (largely free hypoxanthin), on the elimination of purin compounds is illustrated by data cited from a series of experiments by Hilditch, also made at Yale University, in which meat was substituted for the milk and eggs of a purin-free diet. The resulting increase in the excretion of uric acid nitrogen, it is stated, is quite comparable with the figures obtained in the experiment with pure hypoxanthin.

In discussing their work in comparison with that of earlier investigators, the authors point out that the data which they report "emphasize the fact that all of the familiar purins may lead to an increase in exogenous uric acid in the urine of man, with (quantitatively) little influence on the elimination of the purin bases. They may be interpreted to support the most prevalent view that uric acid is a stage in the metabolism of

exogenous purins in the human body, a view rendered especially plausible by the growing statistics on tissue enzyms."

The composition of dilute renal excretions was studied by A. B. Macallum and C. C. Benson (Jour. Biol. Chem., 6 (1909), No. 2 pp. 87–104), the general conclusion being that the elimination of water, potassium salts, and chloride from the body is not due to filtration, but in the case of water, to the physiological activity of the renal membranes involved, and in the case of the salts, to forces which may be termed "secretory."

T. B. Barringer, Jr., and B. S. Barringer (Amer. Jour. Physiol., 27 (1910), No. 1, pp. 119–121) have compared the total nitrogen excretion of either kidney in normal individuals during varying periods of time. In one case the excretions from the 2 kidneys were found to be equal in quantity. Six times they varied by less than 10 per cent and 4 times from 10 to 20 per cent.

"As regards the total nitrogen, in one case the quanitites were equal. In 7 cases they varied by less than 1 gm. per liter. In 2 cases they varied by between 1 and 2 gm. per liter. The nitrogen-urea plus ammonia-urea showed in 3 cases a variation less than 1 gm. per liter and in 6 cases a variation of between 1 and 2 gm."

L. W. Riggs (Abs. in Jour. Biol. Chem., 9 (1911), No. 2, p. XIX; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, p. 13) studied the chemical composition of human sweat, using 45 samples obtained from persons in normal health and from nephritics. The total nitrogen, nitrogen as urea plus ammonia, inorganic acids, potassium, and chlorin were determined in the majority of the samples.

Factors regulating the creatinin output in man were studied by P. A. Levene and L. Kristeller (Amer. Jour. Physiol., 24 (1909), No. 1, pp. 45–65), the experimental data apparently indicating that the formation of creatin and creatinin represents two phases in the catabolism of a single substance. The constant activity of the creatinin output in normal men, the authors believe, is conditioned by the high velocity of creatin combustion in health.

The process of acid excretion was studied critically by L. J. Henderson (Jour. Biol. Chem., 9 (1911), No. 5, pp. 403-424, dgms.

3), who concludes that, as is the case with temperature and osmotic pressure, normal neutrality or alkalinity is adjusted by a mechanism within the body, but is maintained permanently by exchanges with the environment.

In a study of the nutritive value of gelatin, J. R. Murlin (Amer. Jour. Physiol., 19 (1907), No. 3, pp. 285–313) found that under certain conditions, namely, supplying a large proportion of the energy of the ration in the form of carbohydrates being especially favorable, it was possible in experiments with man and dogs to replace part of the proteid nitrogen with gelatin nitrogen for maintaining nitrogen equilibrium at a fasting level.

Gelatin was one of the materials included in a study of the elimination of total nitrogen, urea, and ammonia following the administration of some amino acids, glycylglycin and glycylglycin anhydrid by P. A. Levene and G. M. Meyer (Amer. Jour. Physiol., 25(1909), No. 4, pp. 214–230). The experiments were made with dogs. According to the authors, all of the excessive nitrogen added as gelatin to a standard diet "is eliminated in the form of urea. Thus, this experiment leads to the conclusion that either diketopiperazins do not enter into the composition of the protein molecule, or that the anhydrids of peptids within the protein molecule offer less resistance than when in a free state."

The significance of glycocol and carbohydrate in sparing the body's proteid was also studied by J. R. Murlin (Amer. Jour. Physiol., 20 (1907), No. 1, pp. 234-258). A specific relationship was shown to exist between carbohydrates ingested and the elimination of nitrogen, carbohydrate not needed for combustion being far more efficient for reducing nitrogen output than carbohydrate coming within the requirement for potential energy. This fact, according to the author, indicates the importance of abundant carbohydrates for convalescence and growth, and may explain the almost universal craving for sweets especially in the young.

From experiments with animals on mucic acid and carbohydrate metabolism L. B. Mendel and W. C. Rose (Abs. in Jour. Biol., Chem., 9(1911), No. 2, p. XII; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, p. 6) conclude that this acid "is presumably not an intermediary oxidative product in the metabolism of galactose

or galactose-yielding carbohydrates. The urinary oxalic acid is only very slightly increased after the ingestion of large amounts of mucic acid. This increase is by no means as large as would be expected if mucic acid were a precursor of oxalic acid."

Some experiments on the influence of caffein on protein metabolism of dogs have been reported by W. Salant and I. K. Phelps (Jour. Pharmacol. and Expt. Ther., 2 (1911), No. 4, pp. 401, 402), who also discuss demethylation in the body. The resistance to caffein, the authors state, "was found to vary with the amounts of the urinary purins eliminated."

W. Salant and J. B. Rieger (Jour. Pharmacol. and Expt. Ther., 2 (1911), No. 4, pp. 400, 401) studied the elimination of creatin and creatinin after the administration of caffein, with rabbits. The results indicate that urinary creatin is increased after the administration of caffein, the size of the dose being an important factor. Neither the increased diuresis nor the diminished appetite observed could, in the authors' opinion, be regarded as a factor in accounting for the increased output of creatin.

On theoretical grounds, G. Lusk (Zentbl. Physiol., 21 (1907), No. 26, pp. 861, 862) discusses the specific dynamic effect of portein.

According to H. McGuigan's (Jour. Biol. Chem., 3 (1907), No. 3, Proc., pp. XXXVII, XXXVIII) studies of sugar metabolism in vitro, the clinical assertion is maintained that levulose, is more easily oxidized than glucose and that it may be used in the body when glucose can not. The order of ease of oxidation of a number of sugars is as follows: Levulose, galactose, glucose, maltose, and saccharose, levulose being the most easily oxidized.

H. McGuigan (Amer. Jour. Physiol., 21 (1908), No. 3, pp. 334–350) found that the living muscles of an animal, when perfused with dextrose, levulose, or galactose, caused a rapid oxidation of these sugars. With maltose direct oxidation was not noted. Other questions were also considered in this experimental inquiry, which is a contribution to the question of the way in which the animal body utilizes a carbohydrate food supply.

The income and outgo of nitrogen of a simple mixed diet are determined and briefly reported by Clara C. Benson et al. (Jour. Home Econ., 2 (1910), No. 6, p. 658).

An extended series of studies with fasting subjects has been reported by P. B. Hawk and his associates, in which the following matters have been taken up: Nitrogen partition and physiological resistance as influenced by repeated fasting (Jour. Amer. Chem. Soc., 33 (1911), No. 2, pp. 215–254, dgm. 1); the catalase content of tissues and organs after prolonged fasting (Jour. Amer. Chem. Soc., 33 (1911), No. 3, pp. 425–434); the nitrogen partition of two men through seven-day fasts following the prolonged ingestion of a low protein diet, supplemented by comparative data from the subsequent feeding period (Jour. Amer. Chem. Soc., 33 (1911), No. 4, pp. 568–598); the allantoin and purin excretion of fasting dogs (Jour. Amer. Chem. Soc., 33 (1911), No. 10, pp. 1601–1622); the influence of an excessive water ingestion on a dog after a prolonged fast (Jour. Biol. Chem., 10 (1911), No. 5, pp. 417–432); distribution of nitrogen during a fast of one hundred and seventeen days (Jour. Biol. Chem., 11 (1912), No. 2, pp. 103–127, dgm. 1); the putrefaction processes in the intestine of a man during fasting and during subsequent periods of low and high protein ingestion (Jour. Biol. Chem., 11 (1912), No. 3, pp. 169–177); hydrogen ion concentration of feces (Jour. Biol. Chem., 10 (1912), No. 2, pp. 129–140); on the differential leucocyte count during prolonged fasting (Amer. Jour. Physiol., 30 (1912), No. 2, pp. 174–181); and glycogen-free liver (Jour. Amer. Chem. Soc., 34 (1912), No. 6, pp. 826–828).

Hawk and his associates have also reported an extended series of experiments on the relative effects of copious and moderate water drinking with meals, some of the experiments being carried on as a part of the series of fasting tests referred to above. The following list of subjects studied show the character and extent of the work: The influence of copious water drinking (Univ. Penn. Med. Bul., 18 (1905), No. 1, pp. 7–25); the stimulation of gastric secretion under the influence of water drinking with meals (Jour. Biol. Chem., 9 (1911), No. 2, pp. XXIX, XXX; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, pp. 23, 24); the metabolic influence of copious water drinking with meals (Jour. Expt. Med., 12 (1910), No. 3, pp. 388–410); the uric acid elimination following copious water drinking between meals (Jour. Amer. Chem. Soc., 32 (1910), No. 12, pp. 1686–1691); the excretion of

chlorids following copious water drinking between meals (Reprinted from Arch. Int. Med., 7 (1911), pp. 536-550); intestinal putrefaction during copious and moderate water drinking with meals (Arch. Int. Med., 7 (1911), No. 5, pp. 610–623); the activity of the pancreatic function under the influence of copious water drinking with meals (Arch. Int. Med., 8 (1911), pp. 382–394); the allantoin and purin excretion of fasting dogs (Jour. Amer. Chem. Soc., 33 (1911), No. 10, pp. 1601–1622); the utilization of ingested fat under the influence of copious and moderate water drinking with meals (Jour. Amer. Chem. Soc., 33 (1911), No. 12, pp. 1978–1998); the distribution of bacterial and other forms of fecal nitrogen and the utilization of ingested protein under the influence of copious and moderate water drinking with meals (Jour. Amer. Chem. Soc., 33 (1911), No. 12, pp 1999–2019), a fecal output and its carbohydrate content under the influence of copious and moderate water drinking with meals (Jour. Amer. Chem. Soc., 33 (1911), No. 12, pp. 2019–2032); the influence of an excessive water ingestion on a dog after a prolonged fast (Jour. Biol. Chem., 10 (1911), No. 5, pp. 417–432); the allantoin output of man as influenced by water ingestion (Jour. Amer. Chem. Soc., 34 (1912), No. 4, pp. 546–550); and the hydrogen ion concentration of feces (Jour. Biol. Chem., 11 (1912), No. 2, pp. 129–140).

As a whole the investigations, which are too extended to be quoted in detail, were favorable to the use of water with meals.

The metabolism of inorganic and organic phosphorus was studied with laboratory animals (rabbits) by F. C. Cook (U. S. Dept. Agr., Bur. Chem. Bul. 123, pp. 63, pls. 3), of the Bureau of Chemistry. The rabbits fed organic phosphorus eliminated a smaller proportion of ingested phosphoric acid in the urine than those fed inorganic phosphorus, and the average amount of calcium absorbed from the intestinal tract was higher in the case of the rabbits fed organic phosphorus, the results agreeing with the theory that calcium and phosphorus fed in inorganic form unite to form insoluble calcium phosphate which is eliminated by the bowels in ingested form. The amount of metabolized magnesium that was retained indicates that the rabbits fed inorganic phosphorus, while metabolizing a smaller amount of magnesium

than did those fed organic phosphorus, retained a larger percentage of the amount actually metabolized.

The sulphur balance in metabolism was studied by A. E. Taylor (Abs. in Jour. Biol. Chem., 9 (1911), No. 2, pp. IX, X; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, pp. 3, 4) with 6 normal men, for periods of nearly 3 months. A condition of equilibrium was not observed, the output being regularly and notably higher than the intake. The author does not consider that the results obtained are trustworthy, the presumption being that errors were involved in the determinations of the sulphur index.

According to data reported by A. O. Shaklee and S. J. Meltzer (Amer. Jour. Physiol., 25 (1909), No. 3, pp. 81–112), shaking may completely destroy the three proteolytic ferments—pepsin, rennin, and trypsin. "They are destroyed more rapidly at higher than lower at temperatures; . . . trypsin is more easily destroyed than pepsin; . . . shaking produced by the respiratory movements is capable of causing some destruction of the ferments. Recent experiments by other investigators show also that other ferments may be inactivated by shaking . . .

"The assumption is here made that the nature of the destruction of ferments is similar to that which takes place in the destruction of living cells, and that shaking affects a certain structure which is common to living cells as well as to red blood corpuscles and to ferments."

In experiments on the effects of respiratory movements, ferments in rubber or glass containers of suitable construction were introduced into the stomach and peritoneal cavity, a dog and rabbits serving as subjects.

E. W Rockwood (Proc. Iowa Acad. Sci., 15 (1908, pp. 99–103) has studied the nature of the uric acid ferments which it is believed are concerned in the formation of uric acid from nucleins in the liver.

The problem of nuclein syntheses in the animal body was studied experimentally by E. V. McCollum (Wisconsin Sta. Research Bul. 8, pp. 75–93; Amer. Jour. Phisiol., 25 (1909), No. 3, pp. 120–141) with young and old rats, with normal and special rations, such materials as edestin, zein, glucose, purified butter

fat, and cane sugar being used. These purin-free diets and diets containing purin bases were also compared.

Some of the author's conclusions were as follows:

"The palatability of the ration is a most important factor in animal nutrition. Without palatability the ration may possess all the necessary food ingredients and yet fail to nourish an animal properly. . . .

"Very young animals adapt themselves to a ration possessing a low degree of palatability much better than do adults.

"Other things being satisfactory, all the phosphorus needed by an animal for skeleton, nuclein or phosphatid formation, can be drawn from inorganic phosphates.

"The animal has the power to synthesize the purin bases necessary for its nuclein formation from some complexes contained in the protein molecule, and does not necessarily use purin bases of exogenous origin for this purpose."

A useful summary and digest of data on the elementary composition of nuccleic acids, their constituents, and related questions has been published by P. A. Levene (Jour. Amer. Chem. Soc., 32 (1910), No. 2, pp. 231–240), and a similar compilation on oxidases by J. H. Kastle (Pub. Health and Mar. Hosp. Serv. U. S., Hyg. Lab. Bul. 59, pp. 164).

Methods and standards in bomb calorimetry have been discussed on the basis of experience by J. A. Fries (U. S. Dept. Agr. Bur. Anim. Indus. Bul. 124, pp. 32; Pennsylvania Sta. Rpt. 1909, pp. 321–345).

An adiabatic calorimeter for use with the calorimetric bomb has been described by F. G. Benedict and H. L. Higgins (Jour. Amer. Chem. Soc., 32 (1910), No. 4, pp. 461–467, fig. 1).

The metabolism as a statistical problem has been considered by H. L. Rietz and H. H. Mitchell (Jour. Biol. Chem., 8 (1910), No. 4, pp. 297–326). Such questions as the application of the laws of probability, together with the various mathematical methods of reducing statistical data, the importance of such procedure in metabolism experiments, and related questions are considered.

Comparative physiology of purin metabolism, by H. G. Wells (Trans. Chicago Path. Soc., 7 (1909), No. 8, pp. 244–248; Jour. Amer. Med. Assoc., 53 (1909), No. 21, p. 1741). According to

his summary, "the invertebrates are able to convert adenin into hypoxanthin and guanin into xanthin, showing the presence of the enzyms, adenase, and guanase, but the metabolism proceeds no further. Passing upward in the scale of animal life to the birds and reptiles we find that nitrogen is excreted chiefly in the form of uric acid. Mammals form uric acid only from the purins and have the power of destroying some of the uric acid formed. The enzyms that destroy uric acid seem to be the last formed in development and are possessed by various mammals in varying degrees and in the same animal often show an uneven distribution in the various organs of the body. This uricolytic power is relatively weak in man." The paper is followed by a discussion.

The output of organic phosphorus in urine was studied by G. C. Mathison (Bio-Chem. Jour., 4 (1909), No. 5–7, pp. 274–279) under conditions of work and rest. In young adults, on an ordinary diet, the organic phosphorus was usually found to be more than 0.1 gm. per day. Occasionally it fell below this, while in one case it reached 0.3 gm.

"The percentage of the total P_2O_5 present in organic combination varies considerably from day to day. In the cases examined it averaged 6 per cent of the total.

"The addition of a large quantity of organic phosphorus in the form of glycero-phosphoric acid to the diet had no distinct effect on the output of organic P_2O_5, while it increased the total P_2O_5 output. Glycerophosphoric acid was not broken down by gastric or pancreatic digestion in vitro, so it was probably absorbed unchanged.

"In the observations made, vigorous exercise was not followed by increased output in organic P_2O_5.

"The $N:P_2O_5$ ratio was fairly constant in any one individual on a fairly regular diet. It differed greatly in different individuals, and also in the same individual when the diet was irregular."

The construction of improved forms of respiration calorimeters and progress reports of investigations of the Carnegie Institution of Washington are contained in the reports of the Nutrition Laboratory at Boston, which is under the direction of F. G. Bene-

dict (Carnegie Inst. Washington Year Book, 6 (1907), pp. 130-133; Carnegie Inst. Washington Year Book, 7 (1908), pp. 158–162, pl. 1. dgms. 4).

The respiration calorimeters in use at the Nutrition Research Laboratory of the Carnegie Institution located at Boston, Mass., are described in detail by F. G. Benedict and T. M. Carpenter (Carnegie Inst. Washington Pub. 123, pp. VII+102, pls. 5, figs. 25). A general plan is given of the calorimeter laboratory, the principles involved in the construction of the calorimeter are considered, descriptions of different parts of the apparatus presented, and the calculation of results explained. The descriptions are illustrated with diagrams and reproductions of photographs. An account is given of the routine followed in an experiment with a man as subject. It is hardly possible to give an adequate account of this work in abstract.

Control tests of a respiration calorimeter, by F. G. Benedict, J. A. Riche, and L. E. Emmes (Amer. Jour. Physiol., 26 (1910), No. 1, pp. 1–14), in which alcohol was burned in the respiration chamber and the amounts of heat eliminated, water vaporized, carbon dioxid produced, and oxygen consumed were compared with theoretical values. The results showed that the agreement between measured and theoretical amounts was very satisfactory and that the apparatus has proved as accurate as the usual analytical methods employed in the laboratory, with which small amounts of material are studied chemically or calorimetrically.

The direct determination of oxygen in experiments with the respiration calorimeter has been compared with calculated values by F. G. Benedict (Amer. Jour. Physiol., 26 (1910), No. 1, pp. 15–25), who concludes that the direct determination is accurate, "and that experiments on man can be made in which the direct determination of oxygen is fully substantiated by the indirect determination. Personal experience would indicate that the errors involved in the indirect determination of oxygen are such as to preclude its use under conditions that ordinarily obtain in even the most perfect forms of respiration apparatus, and that accurate determinations of the oxygen consumption of man are practicable only by means of the direct method."

The influence of muscular and mental work on metabolism and the efficiency of the human body as a machine were studied by F. G. Benedict and T. M. Carpenter (U. S. Dept. Agr., Office Expt. Stas. Bul. 208, pp. 100, figs. 3) in connection with the nutrition investigations of the Department of Agriculture, the work being done before the respiration calorimeter was taken from Middletown, Conn., to Washington. The first of the two papers included in the bulletin reports data of 19 experiments on the effects of muscular work on metabolism and the efficiency of the body as a machine, respiratory products and oxygen consumption being measured in the usual way with the respiration calorimeter, and the bicycle ergometer employed for measurements of muscular work. The efficiency of the body was found to be 20 per cent; that is, for every calorie of muscular work produced by the body a total of 5 calories is expended. A series of 44 experiments was made to compare mental work (writing the answers to examination papers which were regarded as difficult by the subjects) and mental idleness, perhaps more properly mental occupation which involved no special mental effort, namely, transcribing an amount of very simple material, which gave them the same amount of arm motion required in writing the examination papers. No constant differences in the heat output in the different periods were noted, so it appears that the experiments do not warrant the conclusion that mental work such as was performed had a positive influence on metabolic activity measurable by the very delicate methods employed.

Metabolism in man with greatly diminished lung area was studied by T. M. Carpenter and F. G. Benedict (Amer. Jour. Physiol., 23 (1909), No. 6, pp. 412–419) at the Carnegie Institution Nutrition Laboratory, a respiration calorimeter being used. The only deduction which can be drawn from the experimental data, according to the authors, is that the reduction of the area for oxygen absorption and carbonic acid elimination in the lungs by about one-half did not materially alter the total metabolism.

During the experiments with the respiration calorimeter made at Middletown, Conn., by T. M. Carpenter and F. G. Benedict (Amer. Jour. Physiol., 24 (1909), No. 2, pp. 187–202) several

cases of illness were observed which, after careful investigation, were attributed to poisoning caused by mercury vapor, due to the use of mercury valves in the ventilating air current.

Metabolism during fever was also studied by the same authors, with the respiration calorimeter (Amer. Jour. Physiol., 24 (1909), No. 2, pp 203–233).

"In general the carbon dioxid excretion was apparently greater during fever than during control periods.

"The oxygen consumption during fever is in practically all cases noticeably greater than during control. . . .

"While the data show a slight tendency for the respiratory quotient to increase during fever, the complications attending the ingestion of food, variations in muscular activity, and errors in oxygen determination do not warrant any sweeping deductions from these data."

The recorded data indicate that in general "there was an increase in the water of vaporization during fever over that during the control period. Since, however, the control experiments showed marked variations when compared with the fever experiments during periods when there was no appreciable fever, it is obvious that here again we can not draw any sweeping deductions regarding this point."

As regards heat elimination, the authors state that "in view of the necessarily tentative nature of all deductions made from these experiments, it has not been deemed advisable to attempt to discuss the influence of fever on the various paths of heat elimination."

The metabolism of man during the work of typewriting was studied by T. M. Carpenter and F. G. Benedict (Jour. Biol. Chem., 6 (1909), No. 3, pp. 271–288), who conclude that "it seems reasonable to assume that the work of writing some 1,500 to 1,600 words per hour on the typewriter results in an increase over the resting metabolism of some 10 to 14 gm. of carbon dioxid, 10 to 13 gm. of oxygen, and 20 to 30 calories of heat per hour. Of these factors of metabolism, it is highly probable that the truest factor is presented by the total energy exchange as directly measured, and hence taking into consideration all the data furnished by these two experiments, we can tentatively say that

the writing of 1,600 words per hour on the typewriter results in a heat transformation over and above the resting metabolism of not far from 25 calories. At present too little is known regarding the energy transformation of various everyday activities to make any striking comparison, but [by other investigation] . . . it has been computed that there is an hourly energy expenditure of about 160 calories over and above the resting maintenance requirement of a man of 70 kg. walking along a level road at a rate of 2.7 miles per hour. It is seen, therefore, that the work of typewriting calls for very much less transformation of energy than does that of ordinary walking."

An apparatus for studying respiratory exchange has been described by F. G. Benedict (Amer. Jour. Physiol., 24 (1909), No. 3, pp. 345–374, figs. 6), which is similar in principle to that portion of a respiration calorimeter which has to do with gaseous exchange and respiratory quotient measurements.

An important digest of data accumulated in experiments with the respiration calorimeter, extending over 10 years, is included in a bulletin on the metabolism and energy transformations of healthy man during rest, by F. G. Benedict and T. M. Carpenter (Carnegie Inst. Washington Pub. 126, pp. VIII + 225). Tentative tables are given for computing the metabolism of normal individuals with varying degrees of muscular activity.

The table on page 217 gives the carbon dioxid eliminated, the oxygen absorbed, and the heat produced per hour during various activities, the data as to standing and very severe muscular exercise being calculated, using as a standard the results obtained with 55 men awake and sitting up.

"The results presented in this report are to be considered simply as indicating the normal metabolism of healthy young men at rest and under several conditions of muscular activity. The variations from the normal exhibited by the individual can be seen by an examination of the tables. The attempt is made to point out the cause of the variations in so far as possible, but with so complex a process as the energy transformation and catabolism in the body, it is clearly futile to attempt to predict with great accuracy either the catabolism or the energy transformations of a given individual. Approximate values that may prove

CARBON DIOXID ELIMINATION, OXYGEN ABSORPTION, AND HEAT PRODUCTION PER HOUR HOUR DURING VARIOUS ACTIVITIES

Degree of Muscular Activity	Number of subjects	Average body weight	Carbon dioxid eliminated	Oxygen absorbed	Heat produced
		Kg.	*Grams*	*Grams*	*Calories*
Man at rest, sleeping.....	17	66.6	23	21	71
Man at rest, awake, sitting up.....	55	64.5	33	27	97
Man at rest, standing, calculated.....	.....	64.5	37	31	114
Man at very severe muscular exercise, calculated ..	.....	64.5	248	213	653

of practical use can be obtained by means of some of the factors outlined in this report. With more accurate and improved calorimeters, there should be in the course of a few years the addition of many factors, at present entirely unknown."

The influence of the preceding diet on the respiratory quotient after active digestion has ceased has been studied by F. G. Benedict, L. E. Emmes, and J. A. Riche (Amer. Jour. Physiol., 27 (1911), No. 4, pp. 383–405).

In general, the conclusion was drawn that the respiratory quotient determined 12 hours after a meal rich in carbohydrates was higher than when the last meal contained only a small amount of carbohydrates. The possibility of this high respiratory quotient being due to the delayed absorption and combustion of carbohydrates in the alimentary tract is discussed, but the authors believe that the evidence is rather against the theory.

"Obviously the previous body condition play a very important role. The extent to which the body storage of glycogen has been drawn upon, the muscular activity of the day previous to the experiment, possibly the temperature of the surrounding air, the general diet of the individual for several days before—in fact, anything which contributes to a disturbance of the storage of glycogen in the body—may alter the influence of the in-

gestion of a carbohydrate-rich meal. If the glycogen storage in the body is at a low point, the ingestion of a carbohydrate-rich meal does not result in an increased respiratory quotient in accordance with the amount ingested, as a not inconsiderable proportion of the carbohydrate may be stored immediately as glycogen. Until this glycogen storage has been replenished the combustion of carbohydrate in the food may be delayed. On the other hand, with individuals subsisting without food and remaining quiet in a respiration chamber, the store of glycogen may last for some time. From these data we may infer, then, that muscular activity may play an important role in effecting the storage of glycogen."

Other questions which have to do with the general subject are discussed.

The respiratory exchange as affected by body position was studied at the Carnegie Institution of Washington by L. E. Emmes and J. A. Riche (Amer. Jour. Physiol., 27 (1911), No. 4, pp. 406–413). In general, the authors found that the pulse rate lying down was on an average 63, the carbon dioxid excretion 209 cc., and the oxygen consumption 236 cc. per minute. With a subject in a sitting position the pulse rate was 71, the carbon dioxid excretion 218 cc., and the oxygen consumption 254 cc. per minute.

In their discussion of the data reported the authors point out that for experimental purposes, when metabolism at a given condition of body rest is to be determined, it is of value to know, "as a result of experiments with the respiration apparatus, that the metabolism of a subject when sitting absolutely quiet in a chair, without extraneous muscular activity, represents a metabolism 8 per cent greater than that of a subject lying on a couch, with similar muscular rest. The difference in metabolism is then due, primarily, to the difference in the internal muscular activity necessitated by the sustaining of body parts. This is in conformity with the well-known fact that the pulse rate of an individual when sitting is always noticeably higher than when he is lying down. From these tests we could infer that if it were possible to so support the body of the subject in a sitting position that the pulse rate would be no greater than when the subject

was lying down, the metabolism would be essentially the same in both positions."

Using a small apparatus for studying respiratory exchange, G. Lusk (Amer. Jour. Physiol., 27 (1911), No. 5, pp. 427–437) investigated the influence of cold baths on the glycogen content of man.

"Immersion of normal men in cold baths at a temperature of 10° when the intestine is free from carbohydrates induces shivering, which causes a rapid utilization of body glycogen, as determined by a fall in the respiratory quotient to the fasting level. In one very muscular individual this result could not be obtained.

"In one individual in whom the shivering had been severe, a quotient of 0.67 and another of 0.62 were found during subsequent periods of rest, which correspond to those observed during rest after a period of exhaustive exercise (glycogen formation from protein).

"The greatest increase in heat production which was brought about by the cold baths was 181 per cent above the normal. The urine remained free from albumin and from sugar."

A respiration apparatus for the determination of the carbon dioxid produced by small animals has been constructed at the Boston Nutrition Laboratory of the Carnegie Institution of Washington and described by F. G. Benedict and J. Homans (Amer. Jour. Physiol., 28 (1911), No. 1, pp. 29–48, dgms. 2).

The effects on men at rest of breathing oxygen-rich gas mixtures were studied by F. G. Benedict and H. L. Higgins (Amer. Jour. Physiol., 28 (1911), No. 1, pp. 1-28, fig. 1), with normal individuals. The air mixtures contained 40, 60, and 90 per cent oxygen, respectively, and the tests were made in a condition of complete muscular rest 12 hours after the last meal was taken. It was found "that there is no apparent difference between the metabolism as indicated by the gaseous exchange (i.e., the carbon dioxid output, oxygen consumption, and respiratory quotient) and the metabolism when breathing ordinary air; that there is no change in the respiration, either as to character, depth, or frequency, as compared with the same factors when breathing ordinary air; [and] that the pulse rate is lower with oxygen-rich mixtures than when breathing ordinary air; furthermore, that

the higher the percentage of oxygen breathed (up to 90 per cent), the lower the pulse."

Data regarding the relative ease of digestion of cheese as compared with beef have been reported by C. F. Langworthy and R. D. Milner (2. Cong. Internat. Hyg. Aliment. Bruxelles [Proc.], 2 (1910), Sects. 4–7, pp. 249–253). No constant differences in the heat elimination per hour were noted when comparable amounts of cheese and meat (beef) were added to a uniform basal ration. So far as the reported data and results of later experiments not yet reported show, there were no marked differences with respect to ease of digestion of these two staple foods.

The success which attended preliminary experiments with the respiration calorimeter on the possibilities of studying physiological processes by means of the gaseous exchange and heat output led to the construction of an instrument of suitable size for this special work and other related problems. C. F. Langworthy and R. D. Milner (U. S. Dept. Agr., Office Expt. Stas. Circ. 116, pp. 3) have described briefly the new calorimeter of small size and also the large apparatus used for experiments with man, as well as a micro-calorimeter for use in the experimental study of very small quantities. The calorimeters as now installed are equipped with recording and controlling devices of special construction. The devices of this character used in connection with the small respiration calorimeter, designed for the study of vegetable problems, involve much that is new and original, so that the calorimeter is very largely automatic in operation. Important modifications have also been introduced into the construction of the calorimeter itself which make for ease of operation.

The work which has been done thus far with ripening fruit has demonstrated that the respiration calorimeter is fully as well suited to the study of certain fundamental problems of plant life as to the study of similar problems of animal life.

The apparatus can also be used, it seems safe to conclude, in studying such problems as the changes which take place when meat or cheese or other similar products are cured or ripened, and factors which influence these changes; that is, problems which are

of commercial interest as well as of agricultural, domestic, and scientific importance.

The construction of the small respiration calorimeter for use in the study of problems of vegetable physiology is given by C. F. Langworthy and R. D. Milner in a later paper (U. S. Dept. Agr. Yearbook 1911, pp. 491–504).

The respiration of apples and its relation to their keeping quality were studied by F. W. Morse (New Hampshire Sta. Bul. 135, pp. 85–92, figs. 2). The results of experiments in one season, calculated on the basis of 1 kg. of fruit for 1 hour, showed that the average exhalation of carbon dioxid was 18 mg. at summer temperature, 8.1 mg. at cellar temperature, and 2.7 mg. at cold storage temperature (32 degrees). The apples obtained in a second series of experiments were 13.2 mg. at cellar temperature, 21.9 mg. at summer temperature, and 5.2 mg. at cold storage temperature.

The expired breath was studied with reference to the presence of organic matter, by M. J. Rosenau and H. L. Amoss (Jour. Med. Research, 25 (1911), No. 1, pp. 35–84, figs. 5). Using the reaction of anaphylaxis, the authors conclude from experiments in which the liquid obtained by condensing the moisture from the expired breath of man was injected into guinea pigs, that the presence of organic matter in expired breath has been demonstrated.

"The logical conclusion from our results is that protein substances under certain circumstances may be volatile. It seems unlikely that such a complex molecule should possess the power of passing into the air in a gaseous form. The volatility, however, now in question may resemble that solubility which deals with particles in suspension in a physico-chemical state (colloidal suspension). The protein may simply be carried over in 'solution' in the water vapor.

"Our experiments are too few to state that albuminous substances such as egg white, milk, or blood serum in vitro is 'volatile.' However, they are sufficiently suggestive to stimulate further work along this line."

FOODS AND THEIR RELATION TO PROBLEMS OF HYGIENE

The need for clean food is much more generally recognized than was formerly the case. This matter and many others pertaining to food in its relation to hygiene have been studied during the period under consideration in the summary.

Studies of the influence of various dietary conditions on physiological resistance have been reported by N. B. Foster (Jour. Biol. Chem., 7 (1910), No. 5, pp. 379–419). The results perhaps pertain more to pharmacology than to dietetics.

The influence of dietary alternations on the types of intestinal flora was studied experimentally by C. A. Herter and A. I. Kendall (Jour. Biol. Chem., 7 (1910), No. 3, pp. 203–236, pls. 3), who observed a marked bacterial degeneration following pronounced physiological alternations in the flora of the intestines as a result of changes in the diet. Experiments were made with laboratory animals. The authors consider it probable that analogous conditions would be found to exist in man.

The question has been further discussed by C. A. Herter (Internat. Beitr. Path. u. Ther. Ernährungsstör. Stoffw. u. Verdauungskrank., 1 (1910), No. 3, pp. 275–281) in a paper.

The results of an extended study of fecal bacteria of healthy men have been reported by W. J. MacNeal, L. L. Latzer, and J. E. Kerr (Jour. Infect. Diseases, 6 (1909), No. 2, pp. 123–169, fig. 1). In general, they conclude that "the direct quantitative determinations of the fecal bacteria furnish evidence of the extent and nature of the bacterial growth in the intestines. This seems to be a delicate index of intestinal conditions."

Numerous studies on the effect of lactic acid ferments on intestinal putrefaction have been reported, including a paper by Helen Baldwin (Jour. Biol. Chem., 7 (1909), No. 1, pp. 37–48).

Coagulated milk and a large number of other preparations were used by P. G. Heinemann (Jour. Amer. Med. Assoc., 52 (1909), No. 5, pp. 372–376) in a study of lactic acid as an agent to induce intestinal putrefaction.

The results obtained led the author to conclude "that so far as the therapeutic effect is concerned, there is yet no convincing evidence that sour milk prepared with commercial cultures is

preferable to naturally soured milk. Yeasts were present in all but one of the commercial preparations."

The bacterial condition of protected and unprotected foods in restaurants, meat markets, grocery stores, bakeshops, and fruit stores was studied by H. E. Barnard (Ann. Rpt. Bd. Health Ind., 27 (1908), pp. 517–523, pls. 4), who found that foods kept in glass cases were in every case practically free from dust and accompanying bacteria, while food on exposed tables and racks was surrounded with air heavily laden with dirt and bacterial life. It was also found that cleanliness of floors and utensils lessened to a certain extent the number of bacteria present, and that on the contrary counters and stands near sidewalks are always surrounded with atmospheric dust and dirt.

The author's studies were concerned chiefly with the relative number of bacteria found on the culture plates inoculated under different conditions and the types of bacteria were not thoroughly differentiated. He believes that pathogenic bacteria were present.

G. W. Stiles (U. S. Dept. Agr., Bur. Chem. Bul. 136, pp. 53, figs. 15) studied shellfish contamination from sewage-polluted waters and from other sources, the observations made in many localities being supplemented by bacteriological work.

According to the author, "there is undisputed evidence to show that shellfish become contaminated when placed in sewage-polluted water, and that *Bacilluscoli* and *B. typhosus* will survive for variable lengths of time in the liquor and the body contents of such shellfish after their removal from infected water. . . .

"Oyster beds should be protected from every possible source of contamination, and they should be located in water proven to be pure by repeated examinations. . . .

"The practice of floating oysters in water of questionable purity should be absolutely prohibited because of the probability of sewage contamination. . . .

"Like other perishable food products, oysters may become unfit for use if stored or kept under insanitary conditions. This spoilage, however, may take place wholly from the length of time out of water. . . .

"The liquor in the shell surrounding the oysters contains more bacteria than does an equal volume of meat from the same oyster. This liquor, together with any sand in the gills of the oyster, can be removed and the meat chilled at the same time by the use of pure ice and water. This washing process can be done efficiently within 3 to 10 minutes, depending upon the method employed. Oysters should not be allowed to soak in fresh water, as they increase in volume, change in appearance and flavor, and decompose more rapidly than those not soaked.

"[As shown by cooking tests], steaming contaminated oysters and clams in the shell, or cooking them after shucking for 15 minutes at boiling temperature, practically destroys all organisms of a questionable character, but since in practice shellfish are never cooked for this length of time, cooking can not be depended upon to remove this danger. . . .

"The investigations show that vast areas of valuable shellfish grounds in this country are now reasonably free from sewage pollution, but this territory will gradually diminish in size if sewage is not properly cared for in the future. Comparatively speaking, only a small acreage is now subject to serious pollution."

The absorption of aluminum from aluminized food was studied by M. Steel (Amer. Jour. Physiol., 28 (1911), No. 2, pp. 94–102). When alum was administered in aluminum-free foods to dogs or when they were given biscuits baked with alum baking powder, "aluminum in comparatively large amounts promptly passed into the blood.

"Absorbed aluminum circulated freely, but as it did not show any pronounced tendency to accumulate in the blood, its full effects must have registered outside of the circulation."

When aluminum chlorid was administered intravenously, from 5.55 to 11.11 per cent of the aluminum passed from the blood into the feces during the 3 days immediately following the injection. "Whether the aluminum passed directly through the walls of the intestine or was excreted by the liver, or whether both channels (or others) were followed, has not yet been ascertained."

The use of metallic containers for edible fats and oils was studied by J. A. Emery (U. S. Dept. Agr., Bur. Anim. Indus. Rpt. 1909, pp. 265–282), of the Bureau of Animal Industry, with

vessels and sheets of tin plate, galvanized iron, copper, tin, lead, zinc, aluminum, and iron, for the purpose of determining the action of fats and oils upon metals, with particular reference to the utility of these metals as containers.

The work shows that where an increase in the acid content of the fat or oil was noted there was an increase in the solvent action of the oil for metals, particularly where other favorable conditions, such as heat, moisture, and exposure to the atmosphere, were present. With cotton-seed oil, however, an exception was noted, as this oil, when prepared with a corn oil of lesser or approximately the same acidity, showed little or no effect upon metals.

"It [was] demonstrated that zinc, copper, and lead are somewhat readily acted upon, while aluminum, iron, and tin, in the order in which they are named, have offered evidences of higher resisting power and are the metals which would more satisfactorily meet the requirements of both manufacturer and consumer."

Much attention has been given in the United States to the discussion and study of pellagra, on account of its supposed relation to Indian corn. The agricultural aspects of the pellagra problem in the United States were studied by C. L. Alsberg (N.Y. Med. Jour. and Phila. Med. Jour., 90 (1909), No. 2, pp. 50–54). Of important discussions may be mentioned the report of C. H. Lavinder (Pub. Health and Mar. Hosp. Serv. U. S., Pub. Health Rpts., 24 (1909), No. 37, pp. 1315–1321), and the paper on the etiology of pellagra by the same author (N. Y. Med. Jour. and Phil. Med. Jour., 90 (1909), No. 2, pp. 54–58), who has also reported considerable data regarding pellagra and its possible relation to maize according to some recent views (Pub. Health and Mar. Hosp. Serv. U. S. Pub. Health Rpts., 26 (1911), No. 8, pp. 199–208). Particular interest attaches to his discussion of Raubitschek's photodynamic theory that the disease is ascribable to the joint action of a substance present in corn meal fat and sunlight.

W. H. Buhlig (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 417–435, figs. 2) and J. F. Siler and H. J. Nichols (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 437–478, figs. 8) have made exten-

sive studies of the possible relation between corn in the diet and the occurrence of pellagra. Final conclusions were not drawn. In connection with this work some data were reported on experiments in cookery as well as regarding institution dietetics.

Cost of Living and Other Statistical Data

The collection of statistical data has continued as an important part of the general activity in nutrition. Much of the work in the United States has been done under government or state auspices.

A select list of references on the cost of living and prices, by H. H. B. Meyer (Washington: Library of Congress, 1910, pp. V+107), has been published by the Library of Congress.

A large amount of statistical data on wages and prices of commodities has been reported in various Senate documents (Washington: U. S. Senate Select Committee, 1910, vols. 1, pp. 658; 2, pp. III+659-875—Hearings held before the Select Committee of the Senate relative to wages and prices of commodities). Topical digest of evidence submitted in hearings held before the Select Committee of the Senate relative to wages and prices of commodities (Washington: U. S. Senate Select Committee, 1910, pp. XCV).

In connection with the work of the U. S. Census many studies have been reported on the production and value of food products; for instance, those on rice cleaning and polishing, by H. McK. Fulgham (Bur. of the Census [U. S.] Bul. 61, pp. 49-58, dgm. 1), and beet sugar, by Z. C. Elkin (Bur. of the Census [U. S.] Bul. 61, pp. 59-69); slaughtering and meat packing (Bur. of the Census [U. S.] Bul. 83, pp. 7-41; and starch, by R. H. Merriam (Bur. of the Census [U. S.] Bul. 64).

The Bureau of Labor has published a summary of retail prices of food from 1890 to 1906 and discussed the data with reference to the cost of living in the United States (Bur. of Labor [U. S.] Bul. 71, pp. 175-328).

A large amount of data regarding the prices of foodstuffs in different New Jersey cities and towns has been summarized in an article on the cost of living in New Jersey (Ann. Rpt. Bur. Statis. Labor and Indus. N. J., 30 (1907), pp. 141-157).

Data regarding the prices of meat in the United States have been summarized in the Yearbook of the Department of Agriculture, by the Secretary, James Wilson (U. S. Dept. Agr. Rpts. 1909, pp. 15–31; Rpt. 91, pp. 10–24; Yearbook 1909, pp. 15–31).

Much statistical data regarding Hawaiian honey are included in a paper on Hawaiian bee keeping, by E. F. Phillips –U. S. Dept. Agr., Bur. Ent. Bul. 75, pt. 5, pp. 43–58, pls. 6).

Information regarding the cost of living of wage-earners and other similar material, including a paper on the preparation of a rational diet at a reasonable cost, are incorporated in the report of the Committee on Social Betterment, by G. M. Kober (Reports of the President's Homes Commission. Washington, D.C., 1908, [pt. 5], pp. 281, pls. 4; Reprint, pp. 281, pls. 4; see also Alimentation and foods (U. S. Senate, 60th Cong., 2. Session Doc. 644, pp. 121–157).

R. C. Chapin (New York, 1909, pp. 372, dgms. 16) has reported an extended investigation of the standard of living among workingmen's families in New York City, which presents and discusses a large amount of statistical data.

The cost of living in American towns has been exhaustively studied by the British Government (London: Govt., 1911, pp. XCII+533, map 1; U. S. Senate, 62 Cong., 1. Sess., Doc. 22, pp. XCII+533), the results being presented in a report by H. L. Smith which was reprinted by the U. S. Senate, and summarized in publications of the U. S. Senate (U. S. Senate, 62 Cong., 1. Sess., Doc. 38, pp. 74) and of the Department of Commerce and Labor (U. S. Dept. Com. and Labor, Bur. Labor Bul. 93, pp. 500–570).

Included in the study were 28 American towns on or east of the Mississippi, with an aggregate population in 1910 of 15,500,000, in round numbers.

The range of price levels for rents was found to vary greatly, being the highest in New York City. The prices of the principal foodstuffs, such as bread, flour, meat, potatoes, and sugar, did not show great range in the different towns, as was evident from the fact that when each article is considered in its relative importance the lowest level is 91 and the highest 109, with New York midway counting as 100. "If the towns are grouped geo-

graphically the New England and southern groups show the highest food price levels, the Midle West towns the lowest, the position of the New England towns in regard both to wages and rents being here reversed."

As regards retail prices of foods, the conclusion is that the ratio between the United States and England and Wales is 138 to 100.

"One peculiarity shown by the budgets is the comparatively small consumption of baker's bread in the average American working-class family, the consumption being $8\frac{1}{4}$ lbs. weekly per family as against 22 lbs. in the United Kingdom, the place of bread being taken in the United States to some extent by rolls, cakes, biscuits, etc., on which the expenditure is about three times as great as that shown in the average British budget. On the other hand, the consumption of meat is much larger in the United States, and the consumption of vegetables is also larger. The budgets indicate in general that the dietary of American working-class families is more liberal and more varied than that of corresponding families in the United Kingdom."

In addition to general discussions the report contains the details of the family budgets and other statistical data collected.

Conclusion

In the foregoing summary of work in human nutrition which has been carried on in the United States since the Seventh International Congress of Applied Chemistry, the attempt has been made to give some idea of the general condition of nutrition investigations and to cite examples of investigations along the proper lines of work into which the subject naturally divides itself.

That the list of investigations is by no means complete is recognized but it is believed that enough has been brought together to show that progress has been continuous and to make it clear that important contributions have been made not only to the fund of available data of interest to the students of nutrition and to practical workers, but also to methods of investigations as well as to the more important matter of fundamental theories of nutrition.

AN IMPROVED FORM OF RESPIRATION CALORIMETER FOR THE STUDY OF PROBLEMS OF VEGETABLE PHYSIOLOGY

By C. F. Langworthy and R. D. Milner

Nutrition Investigations, Office of Experiment Stations, Dept. of Agriculture, Washington, D. C.

Theoretical considerations regarding ripening fruit led to the attempt to study such questions of plant life by methods which have given good results in investigations of topics pertaining to the nutrition and energy expenditure of man. It was found that ripening fruit (bananas) could be studied in this way, since when they were kept during the active ripening period in the chamber of the large respiration calorimeter described in a publication of the Department of Agriculture,[1] carbon dioxid and water vapor were given off and oxygen was absorbed and heat liberated, all in measurable quantities. In other words, conditions were present which could be studied with great exactness with the aid of this apparatus.

It was furthermore apparent from the results obtained in this preliminary work that many other problems of plant life could be studied by such methods and that results of both practical and scientific value could be secured, since a knowledge of the factors determined is of great importance in the consideration of questions pertaining to vegetable metabolism and to the handling and storage of fruit in the home and under commercial conditions.

The chamber of the respiration calorimeter used for experiments with man is of such a size that it will accommodate seven or more large bunches of bananas; that is, its capacity is too great to make it useful for experiments with vegetable products, except those which can be obtained in uniform condition and in fairly large quantities. Furthermore, the entrance to the respiration calorimeter and all its internal arrangements are designed with

[1] U. S. Dept. Agr. Yearbook 1910, p. 307.

respect to experiments with man and are not particularly well suited to experiments with fruits or similar products. It was obvious, therefore, that a smaller respiration calorimeter with special equipment suited to experiments with plant products would be a great convenience and in view of the fact that it would be useful in the study of problems of interest to the Department of Agriculture, such an instrument was constructed. In plan and principle it corresponds to the large respiration calorimeter used for experiments with man although some improvements in grouping of accessory apparatus have been introduced and some new automatic regulating devices which greatly lessen the labor of conducting the experiments have been added, which make for greater accuracy as well as ease of operation.

A respiration calorimeter is thus designated because such an instrument combines in the same device a respiration apparatus for the determination of gaseous exchange of the subject in the respiration chamber during a given period, and a calorimeter for measuring heat liberated in the respiration chamber. Though there are conditions under which it is convenient to use the apparatus either as a calorimeter only, or as a respiration apparatus only, the two operations are usually carried on simultaneously. The two functions of the apparatus, however, are perhaps best considered separately.

The Apparatus as Used for Studying Respiratory Exchange

The chamber of the apparatus is 18 by 18 by 36 inches inside measurements, the walls being copper attached to a wooden framework, and double for purposes which are explained later. The top of the chamber has a cover that may be lifted off so that material may be put inside. The edges of the cover are formed so as to fit into grooves in the upper edges of the sides, and by means of a special wax (mixture of beeswax and Venice turpentine) pressed into the grooves the cover is sealed tight. In the upper part of each of two opposite sides is a window 6 inches by 8 inches sealed into its frame, which forms part of the copper wall. These afford opportunity to observe the fruit during ripening, or to remove a sample if desired. In the third wall is an "outlet," likewise sealed air-tight, through which pass two $\frac{3}{8}$ inch pipes for

the passage of air into and out of the chamber. On the inside the pipe carrying the ingoing air opens near the top of the chamber, while that carrying outgoing air is extended to the bottom, so that air passsing from one opening to the other must traverse the chamber.

The chamber is of sufficient size to accommodate a large bunch of bananas suspended from a framework supported on brackets in the corners near the top; and other brackets have been attached at different levels so that trays, shelves, or other supports may be used. Thus it is possible to place under investigation, at different times, not only a bunch of bananas, but also larger or smaller quantities of apples, or potatoes, or other materials. This material may be packed in the calorimeter in a manner approximating commercial conditions, or, if desired, in some other manner.

On the outside the pipe for outgoing air is connected with a rotary compressor, operated by a small electric motor which withdraws the air from the chamber and forces it any desired rate through a purifying system of special gas washing bottles arranged in series. The first two bottles of the series contain sulphuric acid, which removes from the air all water vapor brought out by it from the chamber. The next two bottles contain granular soda lime (a mixture of caustic soda and quick lime) which removes from the air all the carbon dioxid carried out of the chamber. Following these is another bottle of acid which catches moisture imparted to the dry air by the moist soda lime. The final bottle in the series contains granular sodium carbonate, which catches any sulphuric acid vapor or spray that might be carried from the preceding bottle. The air then returns to the chamber through the pipe for ingoing air. Just before entering the chamber oxygen may be admitted to the ingoing air to replace that used by the material in the chamber.

A small copper pipe, from the side of the chamber, is connected outside with a rubber bag or a spirometer or similar device, the purpose of which is to indicate the volume at any given time, and to maintain atmospheric pressure within the chamber.

The different bottles of the purifying system are weighed on a large sensitive balance to an accuracy of 0.05 of a gram. The

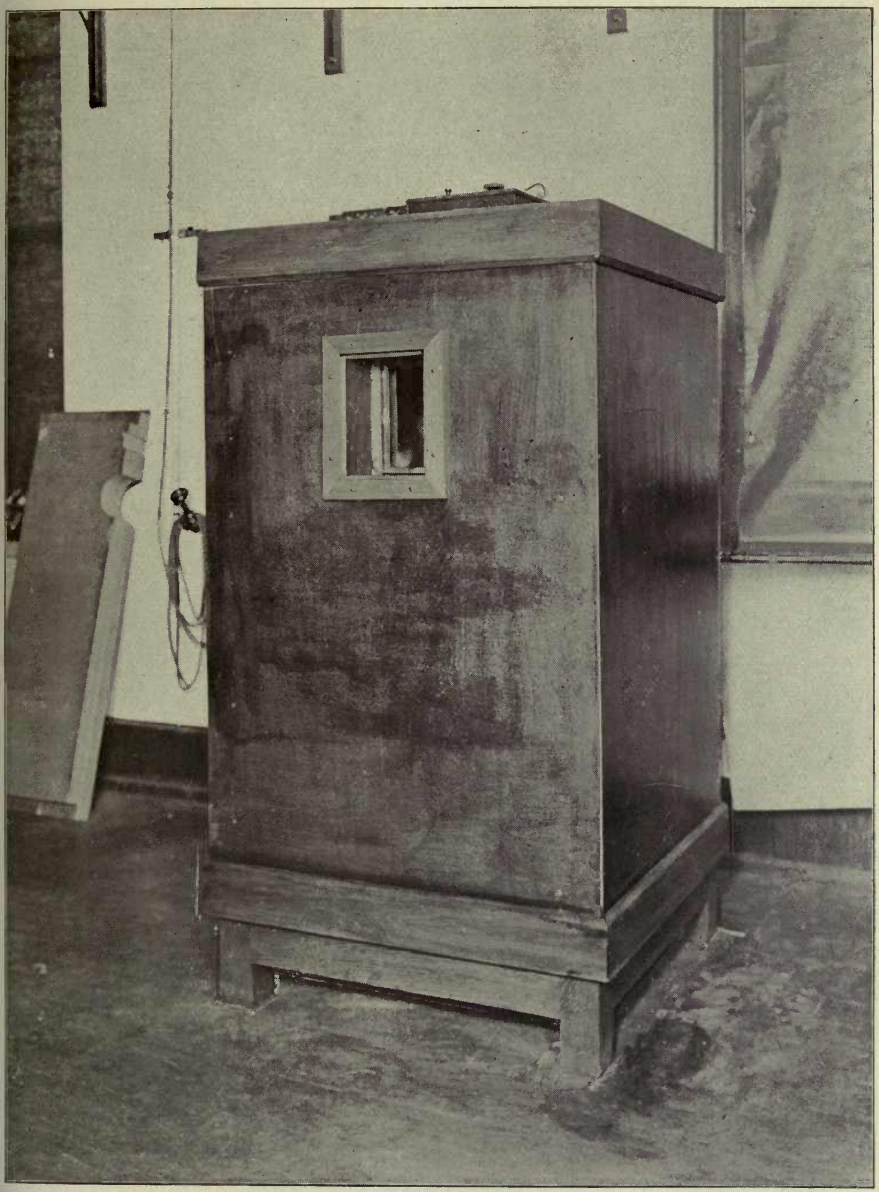
increase in weight of the first two, which contain sulphuric acid, shows how much water was removed from the chamber in the air current during a given experimental period, and that of the two soda lime bottles and of the acid bottle following them shows the amount of carbon dioxid brought out. Under ordinary conditions, in an experiment with fruit, these would represent the amounts actually produced during the period, though whenever necessary samples of the air may be analyzed to determine the amounts residual in the air of the chamber, and allowance may be made for any difference in these amounts at the beginning and the end of the period.

With a ventilating system such as described above, as rapidly as any gaseous substance is removed other gas must be introduced to maintain atmospheric pressure in the chamber. Ordinarily, oxygen is admitted, since oxygen is utilized by the ripening fruit or other material from the air of the chamber. In case the fruit were ripened in some inert gas, such as carbon dioxid or nitrogen, this gas would be admitted instead of oxygen. The gas admitted to the chamber is drawn from a supply under pressure in a steel cylinder that is suspended on a sensitive balance, and the amount admitted is determined very accurately by weighing. In the case of oxygen, for example, from the change in the weight of the cylinder and in the amount residual in the air at the beginning and the end of the period, the amount used by the fruit during the period may be ascertained.

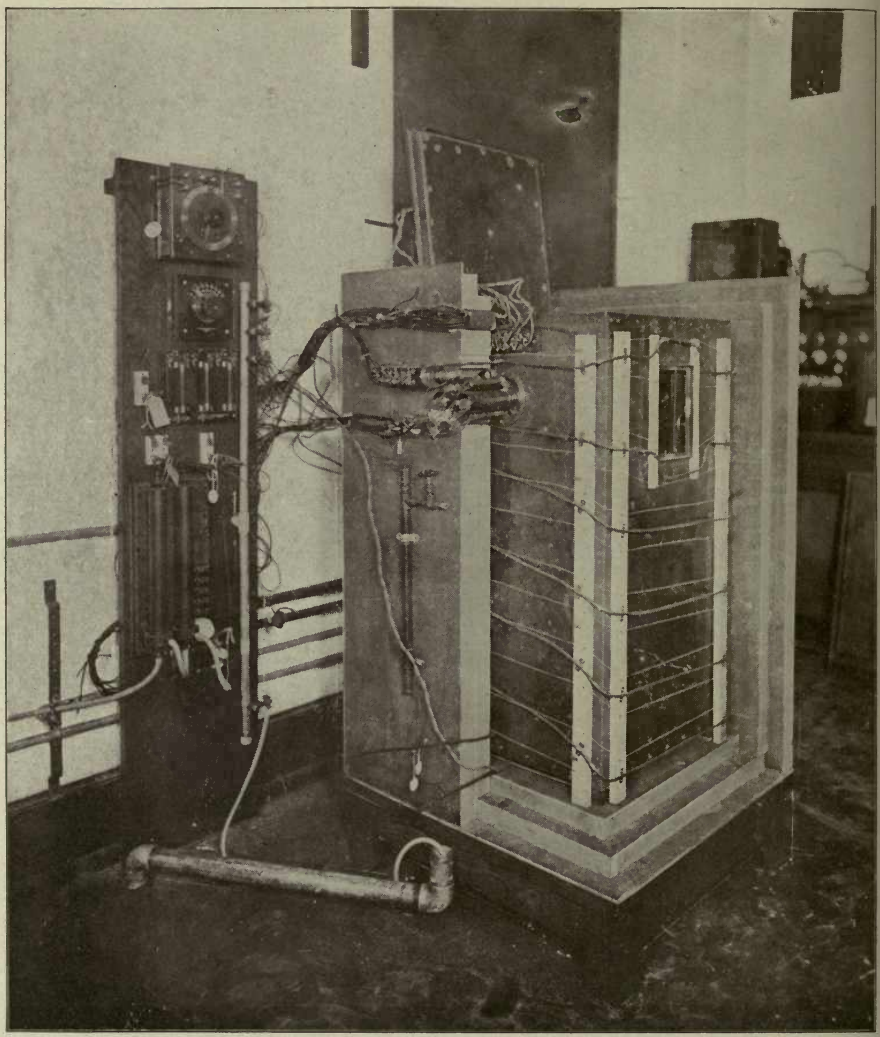
Experimental periods may be of any duration. Since the ripening process continues for several days in the case of bananas, it has been sufficient in these experiments to weigh the different bottles in the purifying system once a day.

The Apparatus as a Calorimeter

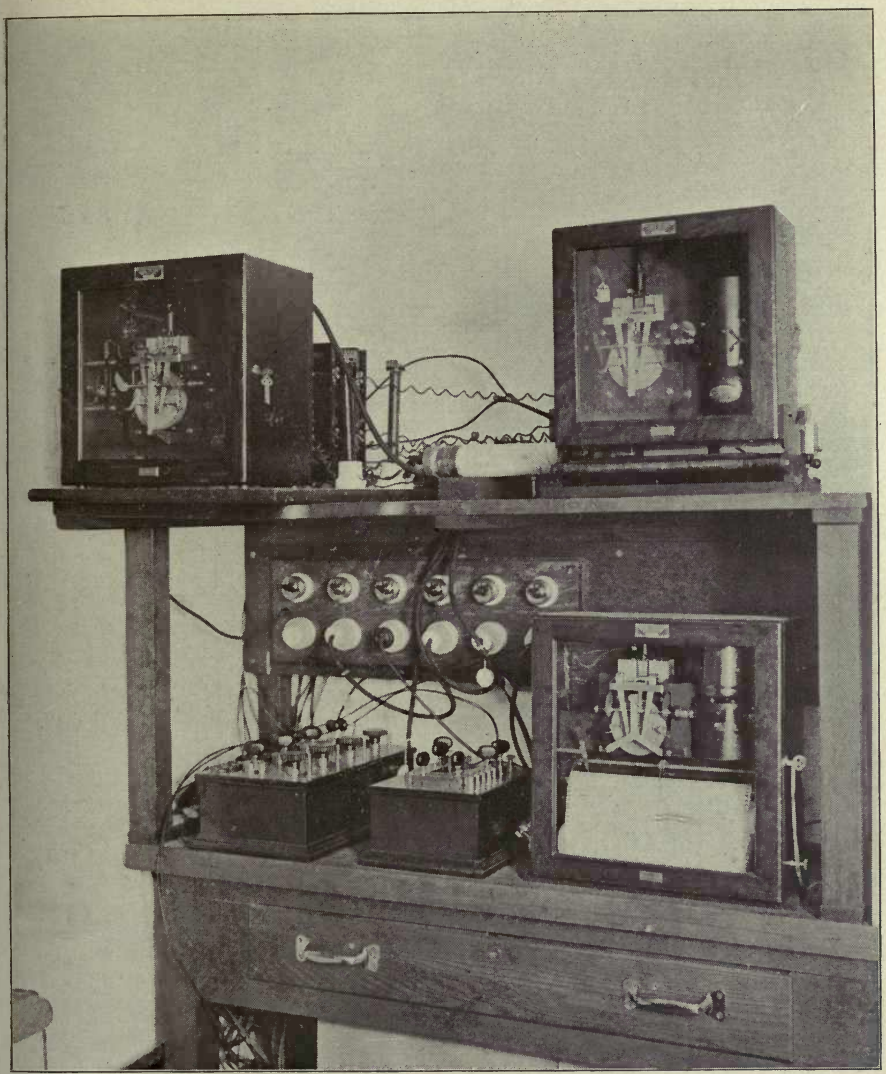
The heat generated by the ripening fruit or other material in the respiration chamber is carried out by a current of cold water flowing through a coil of copper pipe. From the weight of the water flowing through the coil during a given period, and the mean temperature difference between the incoming and outgoing water during the period, the quantity of heat carried out is determined. This quantity and that carried out as latent heat



Photograph No. 1.—This is a view of the exterior of the respiration calorimeter for the study of vegetable problems. The protective covering is in place and the window in one side is shown.



Photograph No. 2.—This is a view of the respiration calorimeter showing how portions of the protective covering may be removed. The cold-water pipes for cooling the air in the space between the protective covering and the walls of the respiration chamber are also shown. The cover of the respiration chamber is raised. On the wall panel are shown switches for various electric currents, the preheater for warming, and the bridge for determining the temperature of the water entering the heat absorbing system, and other devices.



Photograph No. 3.—In the lower right-hand corner of this photograph is shown the automatic temperature recording device with its pen which draws the line representing temperature differences. Next to this on the left is shown the bridge pertaining to this device, by means of which it is possible to vary the range of the records and to test the accuracy of the recording device. Immediately above the recorder is shown a device for the automatic control of the temperature of the water which enters the heat absorbing system in the respiration chamber. At the left on the same shelf is a device which automatically controls the heating of the air adjacent to the top, sides, and bottom of the exterior wall of the respiration chamber and of the ingoing air in the circulating air system. The bridge for this device is shown on the shelf immediately below it.

of water vapor in the out going air represent, with small corrections for changes in temperature of the chamber and of material inside, the amount of heat generated by the material in the chamber.

The quantity of water that flows through the coil in the chamber is ascertained by weighing. Water is kept flowing through the coil within the chamber at as constant a rate as possible. For this purpose the water is drawn from a constant level tank on a shelf above the calorimeter. Since distilled water is used in the circulating system, after it has passed from the calorimeter, it is collected in a tank from which, by means of a small gear pump, it is raised again to the constant level tank. This small pump is driven by the motor which operates the rotary compressor for the circulating air. The purpose of using distilled water in this cooling system is to avoid difficulties due to a presence of air in the water, which tends to collect in bubbles in the pipes and thus form temporary obstructions, causing an irregular flow of water from the heat absorbing system.

The temperature of the air in the calorimeter may be regulated by controlling either the temperature of the water that enters the calorimeter, or its rate of flow, or both. The most convenient practice is to maintain a constant rate of flow and to regulate only the temperature. To this end the water leaving the pressure tank is cooled by passing it through a coil immersed in cold water or brine and then is raised to the desired temperature by means of an electric heating device introduced in the water circuit just before it enters the calorimeter. The ingoing water is kept at such temperature that the heat will be taken up just as fast as it is liberated, so that the temperature of the air in the chamber is kept constant.

This heating device consists of a pre-heater and of a final heater. The pre-heater, which is operated manually, has a total capacity of $5\frac{3}{4}^{\circ}$ C., with a rate of 500 cubic centimeters of water per minute, and heat may be added as needed in small increments. To secure uniformity of temperature, the final heater is separated from the pre-heater by a mixing bottle. The final heater, which is automatic, has a much smaller range than the pre-heater, but it can be adjusted within very narrow limits. The sensitive

portion of the final heater consists of a very delicate electrical resistance thermometer, of a type designed by the National Bureau of Standards, which comprises one arm of a Wheatstone bridge, the slide wire of which is designed to cover by tenths of a degree a range of temperature from 0° to 35°C., shown on the dial of the bridge. This thermometer is placed in the water pipe immediately above a small heating coil of electric resistance wire, so that the water flows from the coil directly over the thermometer. If the temperature of the water flowing over the thermometer differs from the desired temperature, at which the pointer of the bridge is set, the needle of the galvanometer with which the bridge is connected is deflected accordingly; and the automatic controller, of which the galvanometer forms a part, alters the position of a sliding contact on a variable resistance which is in series with the heater just below the thermometer. This results in a change in the amount of electric current through the heater, its heating effect is varied accordingly, and the temperature of the water flowing past the thermometer is regulated until it reaches the desired constant temperature.

The difference between the temperature of the water entering the calorimeter and that leaving is determined by means of a pair of electric resistance thermometers placed in the water line, one just as it enters and the other just as it leaves the chamber, and connected with a Leeds and Northrup temperature recorder. This temperature recorder consists of a self-balancing Wheatstone bridge, two arms of which are formed by the resistance thermometers. The amount of change necessary in the balancing point of contact on the slide wire to balance the bridge at any given time is indicated by a pen that is drawn back and forth on a record sheet that moves forward at a rate of $2\frac{1}{2}$ inches per hour. Since the balance is determined by the mechanism every five seconds, a virtually continuous record of the temperature differences is drawn by the pen. The scale on the paper is 10 inches wide and represents a total range of 2°. The scale is ruled with 100 lines, each representing 0.02°, but the distance between the lines is so wide that 0.01° is very easily read. The bridge part of the apparatus is so constructed that the slide wire may be made

to represent a temperature difference of from 0 to 2°, 1 to 3°, 2 to 4°, or 3 to 5°.

In order that the quantity of heat generated within the calorimeter chamber may be accurately determined, it is necessary to prevent either gain or loss of heat through the walls of the chamber. To this end the respiration chamber has double metal walls and the outer wall is kept at exactly the same temperature as the inner wall, in which case there will be no transference of heat between them. In order to accomplish this it is necessary to provide means for determining any difference of temperature between the two walls and for heating or cooling one wall until it has the same temperature as the other. In this calorimeter the outer metal wall is surrounded by a covering of heat insulating material, between which and the metal wall is an air space about 1 inch across. In this space and surrounding the outer wall of the chamber are a coil of copper pipe to carry cold water for cooling it, and a coil of resistance wire to carry an electric current for heating it. If the outer wall of the calorimeter is too warm it may be cooled by passing cold water through the copper pipe, or if it is too cold it may be heated by passing an electric current through the resistance wire; but in practice it is found most convenient to allow water to flow continually through the coil of pipe and to vary only the heating. This is done automatically.

On the inner and outer copper walls of the calorimeter chamber are electric resistance thermometers which comprise the two arms of a Wheatstone bridge, which have exactly the same resistance when the walls are at the same temperature. As the temperature of one wall varies from that of the other the resistance of the arms of the bridge varies and this causes a corresponding change in a mechanism which controls a variable resistance in series with the heating system surrounding the outer wall, and thus regulates the heating of the outer wall. By this means the temperature of the exterior wall is maintained automatically in balance with that of the interior wall. The controlling mechanism regulates the temperature of the top, sides, and bottom of the chamber independently. The temperature of the air entering the calorimeter is likewise maintained by it

exactly the same as that leaving the calorimeter, so that no heat will be carried in or out in the moving air current.

Possible Uses of the New Respiration Calorimeter

The control experiments and the experimental studies of ripening fruit (bananas) already undertaken have demonstrated the great accuracy of this respiration calorimeter as an instrument of precision and have given interesting results regarding gaseous exchange and heat production which will appear in Department of Agriculture publications.

Although the new calorimeter is arranged with special reference to experiments with fruits and other vegetable products, it is so constructed that the respiration chamber can be removed and another substituted for it of the same size but with different interior arrangements, or of smaller size, should this be desirable. In other words, it would be possible, with little additional labor, since no change in the recording and controlling devices and other accessory apparatus would be involved, to adapt the apparatus to the study of additional problems, such, for instance, as the incubation of eggs and the changes which take place in curing and storing meat products and cheese, or by making suitable provision for the collection of excretory products and for the comfort of the subjects, it would be possible to adapt the calorimeter to experiments with laboratory animals, should the work of the Department make this necessary.

SUR LE ROLE ANTISEPTIQUE DU SEL MARIN ET DU SUCRE

Par M. L. Lindet
Paris, France

Il est facile de concevoir comment certains corps qui sont pour nous des poisons, comme les composés de l'arsenic et du mercure, peuvent arrêter le développement des microbes; mais l'action du sel marin et du sucre, dont nous faisons un usage journalier, me semble ne pas avoir été suffisamment envisagée.

Elle s'explique cependant par la facilité avec laquelle les microbes se plasmolysent; ils cèdent à une solution concentrée de sel ou de sucre une partie de leurs éléments constitutifs, s'affaiblissent, et ne présentent plus la même capacité de reproduction.

J'ai voulu rechercher dans quelle mesure la composition des microbes est capable de se modifier sous l'influence de solutions sucrées ou salines, de concentration variable, et j'ai choisi celui des microbes qu'il est le plus facile de se procurer en masse, la levure de distillerie; celle-ci provenait de la distillerie Springer à Maisons-Alfort (Seine). Dans le but de mesurer la sensibilité du phénomène, je n'ai laissé la levure en contact de la solution que pendant 24 heures, et j'ai dosé l'azote, l'acide phosphorique et la potasse dans les liquides filtrés. J'ai rapporté les chiffres obtenus à la quantité de matières que la levure contenait primitivement:

	Azote	% des éléments contenus dans la levure:	
		Acide phosphorique	Potasse (KO^2)
Témoin Eau pure	1.89	0.57	73.3
Solution de sel à 2%	1.99	1.32	75.4
Solution de sel à 4%	2.19	1.60	77.8
Solution de sel à 8%	2.65	1.77	82.1
Témoin Eau pure	1.89	1.78	73.3
Solution de sucre à 20%	"	5.33	92.6
Solution de sucre à 40%	"	"	93.8
Solution de sucre à 80%	11.13	11.38	96.8

Evidemment les quantités d'azote et d'acide phosphorique dont la cellule s'est appauvrie ne sont pas très considérables, surtout en présence de la solution de sel; mais il faut songer qu'elles représentent les matières les plus solubles de la cellule, celles que la cellule mettra en jeu dès les premiers moments de son évolution. La solubilité des composés potassiques au contraire leur confère un coefficient de diffusion considérable.

L'étude au microscope des levures ainsi soumises à l'action des solutions salines ou sucrées révéle avec netteté leur amaigrissement.

En présence de ces faits, il était intéressant de rechercher comment se reproduisent, sur bouillon de touraillons, gélatiné et sucré des globules de levure qui ont séjourné 48 heures au contact des mêmes solutions. J'ai appliqué, pour la numération des levures la technique que j'ai exposé dans un précédent travail (Comptes-rendus de l'Académie des Sciences, 1910, T. 150, p. 802), et j'ai rapporté le nombre des colonies comptées au mmg. de levure.

Colonies par mmg. de levure:

Témoin	4.514.000
Solution de sel à 5%	4.370.000
Solution de sel à 10%	1.733.000
Solution de sel à 20%	600.000
Solution de sucre à 20%	1.525.000

Il convient en outre de faire remarquer que les colonies de ces différentes levures ont apparu sur la gélatine avec un retard d'autant plus grand qu'elles avaient séjourné au contact de solutions plus concentrées. Une fois apparues, elles n'ont pas augmenté sensiblement en nombre du jour au lendemain; mais celles qui ont été formées au début ont grossi régulièrement, à fur et à mesure qu'elles retrouvaient dans le bouillon gélatine les éléments qu'elle avaient perdus.

J'ai commencé des expériences analogues avec le ferment lactique et avec les champignons; mais ces expériences sont plus difficiles à réaliser, et je demande crédit pour quelque temps.

SALMON CANNING INDUSTRY OF NORTH AMERICA

H. M. Loomis

Chief of the Food and Drug Inspection Laboratory, Bureau of Chemistry, U S. Dept. Agriculture, Arcade Annex Building, Seattle, Wash.

The salmon of the North Pacific Ocean has now become one of the most important marine food products on this continent and its popularity is fast increasing in Europe and other countries. The catching and packing of salmon in the Northwest has developed into such a large industry that it ranks second only to the lumber business. As the growth has been so rapid some apprehension has been felt that the fish might be gradually exterminated but the Federal government and the governments of the various states and of Canada are striving to overcome any such danger by regulating the industry and by establishing hatcheries at various favorable localities.

There are five principal varieties of salmon packed along the Pacific Coast, each one of which is known by several names, depending on the locality where it is caught. The fish with the reddest flesh and most oil are held in the highest esteem by consumers, and in the following list they are given in the commonly accepted order of quality.

1. Red Salmon, Sockeye, or Blueback.
2. Chinook, King or Spring Salmon.
3. Medium Red Salmon, Cohoe or Silverside.
4. Humpback or Pink Salmon.
5. Chum or Dog Salmon.

The 1911 pack of salmon amounted to 290,000,000 cans or six million cases. Of this entire output Alaska produces nearly one half, Puget Sound district about one quarter, British Columbia one sixth, Columbia one twelfth and the balance is caught in various rivers and bays along the coast of California, Oregon and Washington.

For 14 years the United Kingdom has taken an average of over 900,000 cases a year while Australia, the East Indies and South America make continually increasing demands on the product.

The habitat and history of the Sockeye and other salmon is unknown from the time they first reach the sea as young fish until they return to spawn and die—a period of about four years. These fish are not caught by hook and line but in seines and traps of various forms. The traps are located along the shores of the mainland or islands at points which the large schools of fish pass on their journey from the ocean to the rivers, and each trap consists of a row of piling, running out from the shore a distance of several hundred feet. To this, nets are hung vertically to a considerable depth below the water, which depth is regulated by law. These serve to divert the course of the fish into the trap proper, which are rectangular enclosures, formed of nets supported vertically by piles and into which the fish are directed by V-shaped openings. To empty the trap a sort of apron net is raised horizontally until the fish are near the surface and then a bail net is used to transfer the fish to a scow or steamer. As many as 50,000 fish are sometimes taken in a trap at once.

The Sockeye Salmon is comparatively small, weighing from 5 to 10 lbs., while other varieties of salmon are larger—the largest variety, the Chinook, averaging 30 lbs.

Until very recently the older type of soldered can was used for packing salmon but the solderless, so-called sanitary can, is rapidly growing in favor and in 1911 about 1,700,000 cases of fish were packed in the latter form of cans.

To manufacturing chemists it may be of interest to know that in this industry alone 840,000 lbs. of hydrochloric acid, 180,000 lbs. of caustic soda, 6,000,000 lbs. of solder, 137,000,000 sq. ft. of tin plate, and 375,000 gallons of lacquer are used.

Of the 325,000,000 lbs. of salmon caught last year about 225,000,000 were canned and the rest were cured in light brine, frozen, salted or smoked.

I wish to make acknowledgment to "The Pacific Fisherman" for most of the statistics given above.

At the cannery the fish are unloaded and carried by conveyors to:

1. The "iron chink"—a machine which removes the heads, tails, fins, and entrails.

The further steps in the canning process may be briefly enumerated:

2. Cleaning.
3. Washing.
4. Slicing by a machine which cuts the fish transversely into pieces the right size to fit the cans.
5. Adding of salt to cans.
6. Packing of fish in cans. This is usually done by white women in the United States and by Indians in Alaska.
7. Covers put on, crimped and soldered by machine.
8. Cans cooled and vent hole soldered by hand.
9. Cans tested for leaks by immersing in a hot water bath.
10. Placed in steam retort for 1-2 hour.
11. While still hot, covers are punctured, allowing most of the air and some of the liquor to escape. The sound made by striking the can also serves to detect leaks.
12. Cans resealed.
13. Cans heated in steam retorts about one hour at 240° to cook and sterilize contents.
14. Cans are scoured with caustic soda solution and washed.
15. Cans tested for leaks from the sound emitted in tapping the cover.
16. Cans lacquered and labeled.

After the process of canning and before shipment every can is usually tested several times for leaks.

In the United States canneries the labor is almost entirely done by Chinese or Japanese men—the transferring of the fish to the cans employing white women. In Alaska, owing to the scarcity of white labor, most of the canneries employ Orientals and Indians exclusively.

It might be well to mention that the low form of soldered cans, or "flats," are filled by hand, while the tall cans, or "talls," are

filled by machinery and the latter contain a product which is inferior in appearance and price to the former.

In packing the so-called sanitary cans, the number of steps in the process is considerably less and, while the cans are more expensive in the first place, the saving in solder, labor, and other items amounts to about 25c a case. With this form of can there are no vents and the filled cans are run on conveyors through a steam chest, (to heat the contents and expel air), covers are crimped immediately and the cans placed in the retort for final cooking and sterilization.

The methods in use for preparing canned salmon in the United States are generally adapted to the production of a fresh, clean and high grade product.

Since the passage of the National Food and Drugs Act and promulgation of Food Inspection Decision No. 105, regarding the labeling of canned salmon, misbranding is rarely resorted to and the cans are generally labeled to show the variety of salmon contained in them. The public is further safeguarded in the labeling of salmon by the provisions of the Alaska Fisheries Law of June 26, 1906, requiring that in the labeling of canned salmon no false or misleading statement or designation shall be made. The enforcement of this law is in the hands of the Bureau of Fisheries.

A proper study of the composition of canned salmon requires the analysis of many samples of known history, for the different varieties of salmon vary in composition in different parts of the body and at different seasons, the earlier runs of fish being much fatter and finer than the later.

Only a few analyses are reported herewith, but they represent the beginning of a more extended investigation of the composition and quality of fresh and canned salmon at the Seattle Laboratory of the Bureau of Chemistry. The fresh salmon were analyzed within 24 hours after being taken from the traps and were kept on ice as much as possible during that time.

The following notes on the analytical methods seem necessary:

Ammoniacal nitrogen:—For this determination two similar methods were employed. The first method is substantially the alcoholic distillation method of Richardson and Scherubel,

(J. A. C. S., Vol. 30, page 1515), with certain modifications proposed by W. B. Smith. 450 cc. of 95% alcohol by volume were used instead of 60% alcohol, with 25 grams of material and 5 grams of freshly ignited magnesium oxide and the fish was added directly to the distilling flask without previous extraction. 750 cc. Kjeldahl flasks were used and three 150 cc. portions were distilled into N–10 acid, making up the volume in the distilling flask to 450 cc. with 95% alcohol between each portion. The excess of acid is titrated with N–10 caustic soda and cochineal indicator.

The second method was to place 25 grams of fish in a 500 cc. Florence flask, add 5 grams magnesium oxide and 100 cc. of 95% alcohol by volume and distill in a current of boiling 95% alcohol vapor, using an apparatus like that shown on page 37 of Gattermann's "Practical Methods of Organic Chemistry." 400 cc. of distillate were collected in N–10 acid and the excess of acid titrated as above.

In both methods blank determinations were made with the reagents and the necessary correction applied.

CANNED SALMON

1911 pack

	WATER	ETHYL ETHER EXTRACT	PROTEIN (N x 6.25)	TOTAL ASH	NaCl.	AMMONIACAL NITROGEN	
						Richardson method	Alcohol vapor method
No. 1. Puget Sound Sockeye Salmon.....	62.44	15.17	20.25	2.50	0.79	0.0403	0.0348
No. 2. Puget Sound Sockeye Salmon.....	61.84	13.74	21.77	2.73	1.10	0.0437	0.0410
No. 3. Alaska Medium Red Salmon....	69.97	7.81	20.40	2.58	1.09	0.04965	....
No. 4. Alaska Chum Salmon....	73.48	2.88	21.33	2.57	0.83	0.0563	0.0557
No. 5. Alaska Pink or Humpback Salmon	74.12	4.75	19.75	1.98	0.50	.0404	....
No. 6. Alaska Red Salmon.....	70.88	5.26	21.79	2.35	0.64	.0455	....

Each sample was average of two or more cans.
All samples, except No. 2, were old form 1 lb. tall cans.
No. 2 was ½ lb. flat cans.

ANALYSES OF FRESH SALMON, EDIBLE PORTIONS

	WATER	ETHYL ETHER EXTRACT	PROTEIN (N x 6.25)	TOTAL ASH	NaCl	AMMONIACAL NITROGEN	
						Richardson method	Alcohol vapor method
Puget Sound Sockeye Salmon, caught May 7, 1912.	67.48	8.86	22.24	1.36	..	0.0121	0.0205
Puget Sound Steelhead or Salmon Trout, caught May 7, 1912.....	67.89	9.39	21.80	1.35	..	0.0135	0.0218

From a comparison of the fresh and canned Puget Sound salmon there is evidently considerable reduction in water content during the canning process. As all samples of canned salmon were in good condition and gave no indication of deterioration as far as the senses could detect it, the results on "ammoniacal nitrogen" are also of interest, being two or more times greater in the case of the canned product than in the fresh fish.

PROPOSED METHOD FOR THE ESTIMATION OF TIN IN CANNED FOODS

By H. L. Lourie

Bureau of Chem., U. S. Appraiser's Stores, New York, N. Y.

Immediately before and subsequent to the issue of Food Inspection Decision 126, which limits the amount of tin in canned foods to less than 300 milligrams per kilogram, it was necessary in the course of routine work in the New York Laboratory, for a large number of analyses for tin to be made.

The first method used was the Munson Combustion Method as given in Bulletin 107, U. S. Dept. Agriculture. This method had to be discarded because of its length, and its doubtful accuracy in the case of canned foods containing salt, as then there was a loss of volatile tin salts during the combustion. It thus became necessary to find a method that would fulfill two conditions:

I. Accuracy: II. Rapidity:

The first method tried was practically that described in the report to the Local Government Board of England, by Drs. Buchanan and Schryver relative to the presence of tin in certain canned foods, published at London, 1908. In this method the organic material is destroyed as in a nitrogen determination by means of potassium sulphate and concentrated sulphuric acid. While this proved accurate enough, it was discarded because of its tediousness and the constant breaking of flasks. I attempted a modification of this method by using potassium permanganate in conjunction with the sulphuric acid. This was not entirely successful because of the large amounts of permanganate necessary and the constant attention it required. Finally a method was tried using nitric and sulphuric acids to destroy the organic matter. This proved successful from the start, not only being rapid, but also yielding practically 100% recovery, in the case of known amounts of tin. The method was developed not only for

canned materials such as fish, vegetables, fruits, etc., but also for foods high in sugars, such as maple syrup, molasses, jam, etc.,

Below are directions for each class:

Directions for Material such as Fish

Place 25 to 100 grams of the well mixed and finely ground-sample (the quantity employed depending upon the amount of fat or oil present) into a kjeldal flask (800–1000 c.c.), and add 25 to 50 c.c. of concentrated sulphuric acid, the amount depending upon the weight of the charge. Place the flask on a hot plate or on wire gauze over free flame; add about 30 c.c. of concentrated nitric acid, raise the temperature to boil and heat till white fumes are generated, then without cooling add 10 c.c. of nitric acid and continue heating as before. Repeat the nitric acid addition until the solution remains clear (usually straw color), after boiling off the nitric acid fumes. The digestion can be easily accomplished in about one hour with three or four additions of nitric acid. Let the solution cool, and dilute to about 400 c.c. with water. Neutralize with concentrated ammonia, transfer the solution to a beaker, rinse out flask with a little concentrated ammonia, add to main solution, make slightly acid with sulphur ric, and saturate with H_2S gas. Let the precipitate settle on steam bath, filter, wash with a little hot water saturated with H_2S, and then dissolve the precipitate in hot yellow ammonium sulphide. Reprecipitate with acetic acid or hydrochloric acid, filter on ashless paper, ignite, moisten with nitric acid, ignite and weigh as stannic oxide. SnO_2.

Directions for Material such as Syrup

Weigh 50 to 100 grams in kjeldal flask (800–1000 c.c.) and add about 100 c.c. of water and 150 c.c. concentrated nitric acid. Boil until all the fumes are driven off, then add a few c.c. more of nitric acid, and boil to see if there is any further action. Repeat addition of nitric acid and boiling until there is no further action. Then add concentrated sulphuric acid, a few c.c. at a time, heating until all the nitrous acid fumes are driven off. When 20 to 25 c.c. of sulphuric acid have thus been added, boil until

sulphuric acid fumes are driven off. Now add concentrated nitric acid, five c.c. at a time, until the solution is clear. Then proceed as in case of canned goods given above.

NOTE: Fifty c.c. of concentrated ammonia will nearly neutralize 25 c.c. concentrated sulphuric acid. Make usual tests for complete precipitation in the filtrate from the first tin sulphide precipitate. In the case of canned vegetables, as high as 100 grams may be taken without using more than 50 c.c. of sulphuric acid. With fish it is best to take as many c.c. of sulphuric acid grams as grams of fish. The rapidity of the digestion depends on the temperature maintained—the higher the temperature, the faster the material is oxidized.

ON THE PREPARATION OF "NATTO"

S. Muramatsu

College of Agriculture, Morioka, Japan

There are several kinds of *natto* prepared in Japan, but here I mean common *natto* which is a kind of vegetable cheese made by fermenting boiled soya beans wrapped in rice straw and set in a warm cellar for one or two days. Thus the product becomes white and mucilageous by the development of bacteria. *Natto* is consumed as an accessory after having been mixed with table salt and several stimulants, amongst others the powdered mustard is preferred. It is chiefly consumed in Tokyo and the north-eastern districts of Japan and for the production of it Aizu is the noted place. It is chiefly consumed in Tokyo in the summer time, but in the north-east during the winter time, as these are rather poor in vegetables at that season.

There exist several studies on *natto* so far as to its constituents and the micro-organisms forming it, but no exact investigation is known of about its preparation. So, its manufacturers suffer under many difficulties of preparing *natto* of good quality; for this reason, I was obliged to make a study of the method of preparing it and several other points. Besides, I think it is very useful to prepare *natto* of good quality and increase its consumption by the people, as it is a very good and economical food stuff, being cheap and containing much protein, especially in our country where rice is the principal food.

I. Soya Beans

Soya beans are the principal raw material of *natto*. There are numerous varieties of soya beans cultivated in Japan, which, for instance, we can distinguish by their color as yellowish white, green, black, spotted, etc. I prepared *natto* with these different kinds and could not find a more suitable kind than the small yellowish white bean.

The beans which serve for the preparation of *natto* are first sorted and all that are broken or imperfectly developed are picked out; besides, it is better to sift them through sieves with proper meshes to separate too small or too large ones. They are then washed and allowed to steep in clean water for several hours, after that they are boiled in a large iron kettle with sufficient water for ca. 5 hours. Thus the beans become moderately soft and their color darker.

Their constitution was as follows:

	In 100 pts. air-dry beans:—	
Moisture	7.14	
Dry matter	92.86	
	In 100 pts. dry matter:—	
Crude protein	50.156	
Crude fat	22.453	
Crude fibre	6.420	
N-free extract	11.871	Soluble in water 4.329 Insoluble in water 7.542
Ash	3.600	
Total-N	8.025	
Albuminoid-N	7.953	Soluble in water—trace Insoluble in water 7.953
Non-albuminoid-N	0.072	

II. Rice Straw

Rice straw is used for the wrapper of the boiled soya beans. Fresh straw is preferable to old, as its smell is better than that of the latter. The straw is cleaned by taking the muddy leaf away from the under part of the stem and washed with clean water; afterwards it is well tied at its two ends, leaving several inches apart and bundled after filling the bag with the beans. As to the reason of using straw for the preparation of *natto*, it was considered that the straw supplies the proper bacteria to the beans but I do not think this the sole reason, for we can prepare it another way, as, for instance, by setting it in a sterilized Petri-dish or in a basket. When it is made in a basket, which after filling it with beans is put in a warm cellar covered with a straw

mat, it is called basket-*natto*. From this and other facts it is reasonable to consider the principal objects of using straw for the preparation of *natto* to be:—

1. To supply the good aroma of straw to *natto*.
2. To take away ammonia from *natto*.
3. To offer good ventilation of air to the loosely packed beans.

The bacteria which produce *natto* from soya beans are always present on the surface of the beans and their spores being very strong against high temperature, they are not easily killed by boiling, as we can see from the following experiment: The grains which were boiled for several hours are taken in sterilized Petri-dishes after each hour and placed in the incubator at 42°C. By this means, I found that the beans which were boiled for 8 hours become *natto* rich in mucilage and with good aroma.

The fact that the basket-*natto*, which does not come in touch with straw, does not sell as well as common *natto*, for, when we prepare it in the straw bundle its flavor is always superior to one which is made in Petri-dish, as it contains an aroma somewhat like that of straw. So I think that the straw which is used as a bag for the beans gives its good aroma to *natto*.

When the bacteria grow on the beans they produce so much ammonia that we can perceive it by its peculiar smell. As the straw absorbs ammonia, the smell of it is more feeble when we use straw bundle than in the case of glass dish. We can understand this fact when we see that the straw which has been used as a bag always contains much more ammonia than the same fresh material, and *natto*, made in the dish is richer in it than that from bundle.

	Amount of ammonia
In the fresh straw	0.035%
In the straw used as wrapper	0.065%
In *natto* made in a glass dish	0.235%
In *natto* made in straw bundle	0.188%

For these reasons, *natto* prepared in straw bundle must have better flavor than any other, by taking its flavor from straw and giving off the disagreeable smell of ammonia to straw.

The bacteria producing *natto* want much oxygen for their proper growth, as it is an obligate aerobe. So, when we prepare it by heaping up many bundles the interior ones become inferior in quality and also the interior beans of a large bundle become not so viscous as the outer parts. For this reason, it is recommendable to use small bundles for the preparation of superior *natto*.

III. Cellar

The cellar for the preparation of *natto* is made with bricks or with pillars surrounding them with thick layers of straw and plastering the walls with mud; the entrance is furnished with a thick door preventing the entering of air. Along the inside of the wall a long shelf two feet wide is set up at the height of ca. two feet and one or two large hearths are made on the floor for the purpose of warming the room.

IV. The Preparation of Natto

For the preparation of *natto* the soya beans are sorted at first and all beans that are broken or imperfectly developed are picked out. After washing with clean water, they are soaked for several hours and boiled in an iron kettle until they become moderately soft (ca. 5 hours). The boiled beans are put into the straw bundle while they are still hot and the bundles are placed, standing obliquely, on the shelf in the cellar, which is previously warmed by charcoal to about 40°C. The cellar is then shut up carefully, avoiding the ventilation of air; thus, the beans become *natto* after one or two days and are ready for consumption.

V. The Microbes of Natto

As to the micro-organisms of *natto* several authors have made investigations. Dr. Yabe isolated three species of micrococci which formed yellow, orange, and white colonies respectively, and a bacillus which is not motile, liquefying gelatine and producing a greenish fluorescence. He attributed the production of the characteristic aroma of *natto* to the development of the micrococcus which produces yellow colonies; but no explanation was given about the formation of the viscous substance.

Dr. Sawamura isolated various kinds of bacilli and micrococci from *natto* and regarded the following two bacilli as the chief microbes for the production of *natto*.

Bacillus No. 1. is a motile and facultative aerobe. *Natto* produced by this bacillus had a good taste and aroma, but its viscosity was not so great as that produced by the other. The author gave the name of *Bacillus natto* to this bacillus, considering it as the chief microbe in the fermentation.

Bacillus No. 2 is a rarely motile and facultative aerobe. *Natto* produced by this bacillus showed a stronger viscosity but a less nice taste and aroma than that produced by the B. *natto;* he recognised it as a variety of *Bac. mes. vulgatus.* Thus, he concluded that for the formation of good *natto* both bacilli must be present.

Mr. Monzen isolated several kinds of bacteria, among them one bacillus to which Dr. Ōmori gave the name of *Bacillus viscosus natto* and which he said, is the principal microbe that produces strong viscosity. The two kinds of bacilli which he named *Bacillus odorans natto* I and *Bacillus odorans natto II*, produce good aroma in *natto;* and another one which he named *pseudomonas odorans natto*, produces also good aroma. The latter three did not produce good *natto*, unless the material is inoculated also with *B. viscosus natto.* Thus the author concluded that there are necessary for the preparation of *natto* at least two kinds of bacteria, one producing the peculiar aroma and the other strong viscosity.

Mr. Muto isolated several bacteria and concludes that only one bacillus belonging to B. subtilis group is necessary for the production of natto.

I isolated also several bacteria from *natto*, prepared in Tokyo, Aizu, and Morioka, and found that these all contain the same micro-organisms, amongst which the following three bacilli are the principal ones; several other bacilli are not suitable for the preparation of *natto*, as they produce bad color or smell and make the *natto* unfit for eating. Two micrococci were found, one of which was analogous to Mic. flavus, and the other producing a translucent colony on agar plate-culture; but, both the micrococci having no relation to the preparation of *natto*, I gave up their further investigation.

Bacillus No. 1

This bacillus develops most energetically at high temperature (40—50° C.) and produces the best quality of *natto*, providing much mucilage and good aroma.

Form:

The cells grown in bouillon at 40° C. are 1 μ thick and 5-8 μ long.

It moves energetically, providing long cilia around its body.

Spore:

An oval spore is formed principally in one end of the cell, which is 0.8 μ thick and 1.6 μ long; the formation of spore requires 4 hours at 42° C. and germination of it begins equatorial after $2\frac{1}{2}$ hours at the same temperature.

Oxygen:

Obligate aerobe.

Coloring:

It is colored readily with aniline coloring matters and also after Gram's method.

Bouillon culture:

Bouillon remains almost clear after its development, and a strong folded film, colored slightly grayish brown, is formed after 10 hours at 38° C.

Sugar bouillon becomes slightly turbid changing its reaction to acidic at the beginning, which turns alkaline gradually; gas is not formed.

Peptone-water culture:

It produces a grayish white film on its surface and the liquid becomes slightly turbid.

Gelatine plate-culture:

Small white colonies are formed which liquefy it quickly.

Gelatine stab-culture:

It develops vigorously at the surface and liquefies gelatine in the shape of a funnel; the liquefied part remains transparent and a film is formed.

Agar plate-culture:

White and mealy-looking colony, that has a rough wristle at its centre but delicate at its edge, spreading very rapidly at 40° C.

Agar slope-culture:

Colony develops along the line and spreads rapidly all over the surface with mealy appearance; the condensed water remains transparent with a film on its surface, but no sediment.

Potato culture:

Elevated colony is formed in the beginning, which spreads soon over the whole surface of the medium; the colour of the colony is yellowish brown and it is folded with mealy appearance, the medium becoming brown.

Milk culture:

It is coagulated at first and is dissolved again.

H_2S:

Is formed.

Indol reaction:

Is not obtained from old bouillon culture.

Reducing property:

It reduces methylene blue in bouillon but does not develop in the glucose nitrate medium.

Ammonia:

Is formed in the culture of bouillon and Soya beans.

Enzym:

Diastase and proteolytic enzym of tryptic nature are recognised.

Behaviour to temperature:

It develops very vigorously at 50° C., but not at 60° C. It is killed at 60° C. after two hours, and after one hour at 80° C.

The resistance of the spores against heat is very strong, for it wants one hour to be killed in Koch's steam-steriliser.

Behaviour to several compounds:

Table salt:

In bouillon containing 15% NaCl it develops slowly, but not in 20%solution.

Alcohol:

It develops in bouillon containing 4%, but not in 5% alcohol.

The spore is not killed readily with alcohol, as it is yet alive after ten days and more, when put either in 50% or absolute alcohol at 20° C.

HCl:

It develops in bouillon containing 0.025% HCl, but not in 0.05% The spore which is put in 3% HCl is alive after one day, but not

after two days. In 4% HCl it is alive after one hour, but not after two hours.

Acetic acid:

It develops in bouillon with same concentration as hydrochloric acid.

The spore is not killed by glacial acetic acid after 10 days and more.

NaOH:

It develops in bouillon containing 0.2% NaOH, but not in 0.3%.

The spore is killed when it is put in 35% solution after one day.

Phenol:

It develops in bouillon containing 0.1% Phenol, but not in 0.2%.

The spore is not killed after ten days when it is put in 5% solution.

Corrosive sublimate:

It develops in bouillon containing 0.0025% $HgCl_2$, but not in 0.005%.

The spore is killed after 50 minutes when it is put in 0.1% solution, but it was alive after 40 minutes in the same solution.

This bacillus may be the same as those which Dr. Sawamura represented as *Bacillus No.* 2 and *Bacillus viscosus Ōmori*, and also that which Mr. Muto thought was the only bacterium which produces *natto*, though there are several differences in its behaviour investigated by these authors.

Bacillus No. 2

This bacillus develops most energetically at high temperature and produces *natto* of the best quality, forming much mucilage and rather higher aroma than Bacillus No. 1.

Form:

The cells grown in bouillon at 40° C. are 0.8-1 μ thick and 4-10 μ long.

Motility:

It moves vigorously providing long cilia around its body.

Spore:

An oval spore is formed in one end of the cell, and it is 0.8 μ thick and 2 μ long.

The spore wants 4 hours at 42° C. for its formation and it germinates equatorial after $2\frac{1}{2}$ hours at the same temperature.

Oxygen:

Obligate aerobe.

Colouring:

The cell is coloured easily by aniline colouring matters and also after Gram's method.

Bouillon culture:

Bouillon remains almost clear after its development and a strongly folded film of slightly grayish brown is formed after twelve hours at 38° C. Sugar bouillon becomes slightly turbid, changing acidic at the beginning which turns alkaline gradually; gas is not formed.

Peptone-water culture:

It produces a grayish white film on its surface and the liquid becomes turbid slightly.

Gelatine stab-culture:

It develops on the surface quickly and liquefies gelatine in the shape of a funnel; the liquefied part remains transparent and a film is formed.

Agar plate-culture:

There is formed a white and mealy-looking colony with rough wristle at its centre but delicate at its edge, spreads very quickly at 40° C.

Agar streak-culture:

Colony develops along the line and spreads rapidly all over the surface with mealy appearance. The condensed water remains transparent with a folded film on its surface but no sediment.

Potato culture:

Elevated colony is formed in the beginning, which soon spreads over the whole surface of the medium; the colony is folded and has brownish yellow colour and mealy appearance; the medium becomes brownish gray.

Milk culture:

It is coagulated at the beginning and is dissolved again.

H_2S:

Is not formed.

Indol reaction:

Is not obtained from old bouillon culture.

Reducing property:

It reduces methylene blue in bouillon and produces ammonia by reduction of nitric acid in the glucose nitrate medium.

Ammonia:

It is formed in the culture of bouillon and soya beans.

Enzym:

Diastase and proteolytic enzym of the tryptic nature are recognised.

Concerning the behaviour against heat and several compounds as formerly mentioned, there is not much difference with *Bacillus No. 1.*

This bacillus may be the same as that which Dr. Sawamura named *Bacillus natto,* though there are several differences in its behaviour investigated by us. As this bacillus does not produce any mucilage at low temperature (say, 35° C.) he thought it, perhaps, to be one which produces aroma peculiar to *natto;* but, as I mentioned already, this bacillus produces much mucilage at higher temperature and makes good *natto* with high aroma.

Bacillus No. 3

This bacillus develops most energetically at 40° C., and when it is developed on boiled soya beans at this temperature, it produces good *natto* with strong viscosity and good aroma; but its mucilage is somewhat less than *Bacillus No. 1* and *Bacillus No. 2.*

Form:

The cells grown in bouillon at 40° C. are 1.2 μ thick and 6-10 μ long.

Motility:

It moves providing long cilia around its body.

Spore:

An oval spore is formed in one end of the cell, which is 1 μ thick and 1.5 μ long.

The spore is formed after 4 hours at 42° C. and germinates equatorial after $2\frac{1}{2}$ hours at the same temperature.

Oxygen:

Obligate aerobe.

Colouring:

It is coloured readily with aniline colouring matters and also after Gram's method.

Bouillon culture:

Bouillon becomes slightly turbid and a brittle film of slightly grayish brown colour is formed after ten hours at 38° C. and produces a small amount of sediment. The film is broken easily by shaking and sinks to the bottom. Sugar bouillon changes to slightly acidic at the beginning and turns slightly alkaline afterwards.

Peptone-water culture:

It becomes slightly turbid and forms a yellowish white film on its surface. Gas is not formed.

Gelatine plate-culture:

Small white colonies are formed which liquefy it quickly.

Gelatine stab-culture:

It develops on the surface at the beginning and liquefies gelatine in the shape of a funnel, afterwards thoroughly.

Agar plate-culture:

White colony with rough wristle at its centre but delicate at its edge, spreads very rapidly at 40° C.

Agar slope-culture:

The colony develops along the line and spreads rapidly in the shape of a feather; the condensed water is transparent with a film on its surface, but no sediment.

Potato culture:

Yellowish gray colony is formed, somewhat elevated in the beginning; it spreads soon over the whole surface of the medium. The colony has strong viscosity and it is folded shallower than *Bacillus No. 1. and Bacillus No. 2.*, the medium becoming gray.

Milk culture:

It is coagulated and dissolved again.

H_2S:

Is produced.

Indol reaction:

Is not obtained from old bouillon culture.

Reducing property:

It reduces methylene blue in bouillon, and ammonia is formed by the reduction of nitric acid in the glucose nitrate medium.

Ammonia:

Is formed in the culture of bouillon and soya beans.

Enzym:

Diastase and proteolytic enzym of tryptic nature are recognized.

The behavior to heat and several compounds is almost the same as with Bacillus No. 1, although there are some differences.

This may be the same bacillus as *Bacillus grossus*, but as there is no detailed description of it, I cannot make a precise comparison.

VI. The Application of Cultured Bacteria for the Preparation of Natto

As mentioned already, when we prepare *natto* in a glass dish at ca.30°C. inoculated with *Bacillus No. 1* it has some viscosity, while others have not, but the aroma was inferior to that made in straw bundles, for it does not touch with straw. At 45°C. all bacilli produce *natto* of fine quality providing strong viscosity and good aroma; the aroma produced by *Bacillus No. 1* was the best, while *Bacillus No. 2* produces a rather strong smell of ammonia, and *Bacillus No. 3* being the worst; moreover, I prepared *natto* according to the common way differing only on the point of inoculating these bacilli separately and also mixing them with one another. The result was that *natto* which was produced by the inoculation of *Bacillus No. 1* was the best, as it has much mucilage and fine aroma, while *Bacillus No. 2* produced an inferior and *Bacillus No. 3* the worst quality. *Natto* produced by the inoculation of mixed baccilli was not so good as that produced by each bacillus; so, there is no necessity that two or more bacilli present for the formation of good *natto*. By the inoculation of cultured bacteria we can entirely avoid failures and can prepare good *natto* by selecting the bacteria. Otherwise, it is sufficient to put it in the cellar for only one day, after which the *natto* will be ready for consumption. So, I recommend to use the pure culture of proper bacteria according to the following way:

The bacteria developed on the slope culture medium of agar are mixed with juice produced by the boiling of beans. This is poured over the surface of boiled beans while they are still in the kettle, the further process being the same as usual. There is no necessity of mixing several bacilli.

VII. Natto as a Food Accessory

As *natto* is prepared from soya beans which are rich in protein and carbohydrates, it contains much protein and carbohydrates; the nutritive value of it is greater than that of boiled soya beans, for it is rich in soluble matters produced by the micro-organisms.

The composition of *natto* differs exceedingly with age, but its mean composition is as follows: (Compare with the composition of boiled soya beans.)

	In 100 pts. of fresh *natto*.		
Moisture	53.480		
Dry matter	46.520		
	In 100 pts. of dry matter:		
Crude protein	46.088		
Crude fat	20.216		
Crude fibre	6.140		
N-free extract	3.348	Soluble in water	2.495
		Insoluble in water	0.853
Ash	5.010		
Total-N	7.374		
Albuminoid-N	5.458	Soluble in water	1.141
		Insoluble in water	4.317
Non-albuminoid-N	1.916		

The micro-organisms which grow on the soya beans secrete trypsin and diastase; so, when we take it together with several foods rich in protein or starch, they may be digested more rapidly than when they are taken alone.

I express many thanks to Dr. Satō, Director of our College, who helped me in determining the quality of *natto* that I prepared, and also to Mr. N. Nitta and Mr. Y. Tanaka who assisted me in these investigations.

CONTRIBUTION TO THE CHEMISTRY OF THE RIPENING OF "SHIOKARA"

By Y. Okuda
College of Agriculture, Imperial University, Tokyo

Although the isolation and identification of some nitrogenous compounds in "Shiokara" has been undertaken about two years ago by Prof. U. Suzuki, Yoneyama and Ōtake in this laboratory, no chemical investigation about the ripening process of this interesting food material has yet been reported. So I have tried to contribute something on this line. I have observed that the autolysis and the action of microbes are two indispensable factors for the preparation of "Shiokara."[1] Some trials have also been made to isolate the enzymes which play an important rôle in this process, and finally, I have carried out some quantitative determinations to see the chemical changes at different stages of ripening.

I. Autolysis and the Action of Microbes

1). To see whether the autolysis is going on during the ripening process of "Shiokara," very fresh organs[2] of a bonito fish were minced with a meat-chopping machine, and rubbed with some quartz sand in a mortar. 40g of the paste thus prepared was divided into two equal parts, and put in the flasks A and B. After adding 100 c.c. of water to each flask, A was boiled for a few minutes to destroy the enzymatic action. Both flasks were then shaken with enough toluol and a little chloroform, and kept for 4 days at ordinary temperature. No bacterial growth was observed during that time. The flask B was

[1] "Shiokara" made from the organs of bonito was used.
[2] The stomach, the intestines, and the pyloric coecum.

now boiled, and the contents of both flasks were then filtered and analysed with the following results:—

	A (boiled)	B (not boiled)
Total soluble–N	1.697%	1.895%
Soluble Alb.–N	0.184 "	0.141 "
Non-alb.–N	1.513 "	1.754 "

2). The fresh "Shiokara" two days after preparation was chopped and crushed in a mortar, 200g of the paste were divided into two equal parts and put in two flasks of 1 litre capacity, and stoppered with cotton plugs. After adding 500 c.c. water, one flask was boiled. To each flask was now added enough toluol and chloroform and after keeping for 4 days at room temperature, the contents of both flasks were filtered and analysed.

	A (Boiled)	B (not boiled)
Total soluble–N	1.848%	2.052%
Soluble Alb.–N	0.056 "	0.038 "
Non-alb.–N	1.792 "	2.014 "
Amino–N (after formol method)	0.604 "	1.023 "

We see from the above two experiments that autolysis is going on in the fresh organs of bonito fish, and also in the freshly prepared "Shiokara."

3). The microbes, predominating in "Shiokara" seem to be quite different at different stages of its ripening. In three preparations, made in April and two months old, we found immense numbers of yeasts, bacilli and cocci, but only few moulds, while in a sample prepared early in October and about one and a half months old, were found numerous yeasts, the other microbes being relatively very few.

The isolation and identification of these microbes will be reported afterwards.

4). 120g of the "Shiokara", which was two months old, were well crushed and equally divided into three Erlenmeyer

flasks containing each 100 c.c. of saturated natrium chloride solution and treated in the following way:—

A. Control:—Not boiled, no antiseptics added.

B. Not boiled, toluol and chloroform added to prevent the bacterial growth, but not the enzymatic action.

C. Boiled and antiseptics added to prevent both bacterial and enzymatic action.

After keeping for ten days at 34—38°, they were boiled and filtered, and the filtrates were analysed with the following results:—

	A (Control)	B (Not boiled, but antiseptic added)	C (Boiled and antiseptic added)
Total soluble–N.....	2.404%	2.305%	2.305%
Soluble alb.–N.....	0.049 "	0.090 "	0.184 "
Non-alb.–N.....	2.355 "	2.215 "	2.121 "

The above experiment shows that both autolysis and the action of microbes are going on very slowly in the old preparations compared to fresh ones. The investigation of Wehmer[1] on salted herring has shown that the action of microbes upon proteins is somewhat retarded in 5 per cent. common salt solution, but it does not entirely stop even in 30% solution. As the concentration of the salt in "Shiokara" usually is 15%, there is no doubt that the microbes can still play an important rôle on the ripening process, especially at the early stage of its preparation.

II. Enzymes in 'Shiokara"

Trypsin, diastase and lipase were identified in the fresh organs of a bonito fish and also in the fresh preparations of "Shiokara." In the old preparation, however, their action seems to be much retarded. This observation agrees well with the experiments mentioned above.

[1] Wehmer: Abhandlungen des deutschen Seefischerei—Vereins, III, 1898.

1). Fresh organs. The stomach, intestines and pyloric coecum of a fresh bonito were freed from their contents and rubbed with some quartz sand in a mortar, and filtered through the cloth filter. The faintly acid extract thus obtained has shown its peptonifying power upon milk and fibrin, either in the faintly acid reaction or after addition of 0.2% sodium carbonate. But no action was observed in presence of 0.2% hydrochloric acid in the medium, thus the absence of a pepsin is most profitable.

The existence of diastase was shown by the saccharification of starch paste and glycogen in the neutral reaction.

For the detection of lipase, the minced and ground organ was extracted with a mixture of 90 parts of pure glycerine and 10 parts of 1% sodium carbonate, 10 c.c. of the mixture being used for 1 g of the sample. The liquid was filtered through a piece of cloth and exactly neutralized. By the addition of some milk or olive oil to this extract, the increase of acidity due to the formation of fatty acids by the action of lipase upon neutral fats was observed. Of course some toluol and chloroform being added to prevent the bacterial growth.

2). "Shiokara" at different stages of ripening. The following observation was made with the samples collected at different stages of ripening:—

(a). "Shiokara," two days old.
Trypsin. Present, active.
Diastase. Do.
Lipase. Present, but the action was very weak.

(b). "Shiokara." 40–50 days old.
Trypsin. Present, but very weak.
Diastase. No reaction.
Pepsin. Do.

(c). "Shiokara," 50–60 days old.
Trypsin. Very weak.
Lipase. Do.
Diastase. No reaction.

3). Isolation of enzymes. For this purpose, about 200g of the fresh sample, 3 days old, were finely minced and ground with some quartz sand in a mortar and macerated with a little

distilled water. The liquid was strained through linen cloth, and after dialysing for about two hours to get rid of the greater part of the common salt, it was poured in a mixture of absolute alcohol and ether, the grayish white voluminous precipitate thus produced was then collected on a filter, washed with absolute alcohol and ether, and dried over sulphuric acid. The crude enzyme preparation obtained in this way, when dissolved in a little water has shown strong diastatic and tryptic action while that of lipase was very weak. When the solution of this crude enzymes was added to a solution of various amino-acid, no liberation of ammonia was observed, showing the absence of amidase.

The proteolytic enzyme which acts in weak alkaline as well as in neutral or in faintly acid reaction, but not in a 0.2% hydrochloric acid solution, was also found by Blanchard[1] in several fishes and by Roaf[2] in two crustaceae.

[1] Blanchard, Jahresbericht für Their-Chemie, 13, 1883—Orig. Compt. rend. 96, 1241.

[2] Roaf, Jahresber. f. Tier Chem. 36, 1906—Orig. Biochem. Journal, 1. 390–97.

III. Chemical Changes During the Ripening Process

1). The sample[1] used for this determination was prepared on the 17th of June, 1911, and after 3, 6, 12, 25, and 40 days respectively a portion was taken for analysis. Thus the following results were obtained:—

Date of analysis	3	6	12	25	40	Days after preparation
In 100 parts of fresh samples						
Water	64.95	64.78	64.58	64.25	63.99	
Dry matter	35.05	35.22	35.42	35.75	36.01	
Total–N	1.98	2.04	2.05	.....	2.07	
Alb.–N	0.35	0.35	0.28	0.26	0.14	
Ether-extract	1.83	1.81	1.81	1.71	1.74	
Soluble matter	27.25	28.45	29.24	31.10	31.14	
Non-alb.–N	1.63	1.69	1.83	1.98	2.01	
Ammonium–N	0.15	0.15	0.25	0.18	0.13	
Organic base–N	0.84	0.72	0.69	0.63	0.64	
Other–N	0.74	0.80	1.09	1.17	1.43	
Total acid (as lactic)	1.43	1.42	0.97	0.96	0.98	
NaCl (calculated from total chlorine)	17.31	.....	17.34	.....	17.71	
In 100 parts of dry matter						
Total–N	5.64	5.79	5.79	.....	5.76	
Alb.–N	1.00	1.00	0.80	0.71	0.38	
Ether-extract	5.21	5.13	5.10	4.77	4.84	
Soluble matter	77.74	80.76	82.53	86.99	86.49	
Non-alb.–N	4.64	4.79	5.18	5.54	5.57	
Ammonium–N	0.43	0.43	0.70	0.51	0.36	
Organic base–N	2.39	2.04	1.97	1.77	1.79	
Other–N	2.12	2.26	3.09	3.26	3.98	
Total acid	4.07	4.02	2.73	2.67	2.72	
Common salt	49.37	.....	48.95	.....	49.17	

[1] This sample contained the stomach, intestines, pyloric coecum and very little liver.

2). The second sample[1] was prepared on the 3rd of Oct. 1911, and after 1, 14, and 53 days respectively, a portion was taken for analysis:—

	In 100 parts of fresh sample			In 100 parts of dry matter			Days after preparation
Date of analysis	1	14	53	1	14	53	
Water.....	64.39	63.00	60.33	0.0	0.0	0.0	
Dry matter	35.61	36.99	39.67	100.0	100.0	100.0	
Total-N.....	2.29	.....	.....	6.42	.....	.....	
Total acid.....	0.95	1.05	1.14	2.65	2.85	2.88	
Ether-extract.....	6.84	6.89	.....	19.19	18.61	.....	
Soluble matter.....	25.61	27.14	28.90	71.92	73.27	72.86	
Non-alb.-N.....	1.59	1.85	2.07	4.45	4.99	5.21	
Ammonium-N.....	0.10	0.12	0.14	0.28	0.32	0.35	
Organic base-N.....	0.81	0.73	0.71	2.27	1.96	1.79	
Creatinine-N.....	0.01	Trace	Trace	0.02	Trace	Trace	
Creatine-N.....	0.02	Trace	Trace	0.04	Trace	Trace	
Xanthine base-N ...	.....	0.06	0.03	.....	0.16	0.08	
Other-N.....	0.66	1.00	1.21	1.86	2.71	3.05	
Natrium chloride....	13.51	13.94	.....	37.94	37.62	.....	

The results of the above two analyses may be summarized as follows:—

	(1)	(2)
Soluble organic matter	Gradually increased	Do.
Alb.-N	" decreased	.....
Non-alb.-N	" increased	Do.
Ammonium-N	Increased at first and decreased hence-forward.	Gradually increased
Monoamino-N	Gradually increased	Do.
Organic base-N	" decreased	Do.
Creatine-N	.....	Gradually decreased
Creatinine-N	.....	" "
Xanthinbase-N	.....	" "
Ether-extract	Somewhat decreased	Do.
Total acid	Decreased	Somewhat increased

[1] This sample contained more liver than the former one.

Thus the results of two analyses resemble each other in general respects, only the contradictory results were observed with ammonia and with total acid. This may be due to the differences of materials and also the temperature during the experiments.

3). I will add here some qualitative tests made about the distillates obtained by the steam distillation of two shiokara-preparations, in neutral as well as in acid reaction.

In the distillate	10 days after preparation	61 days after preparation
Alcohol	(+)[1] (very little)	(−)[1]
Aldehyde	(−)	(−)
Acetone	(−)	(−)
Indol	(−)	(−)
Phenol	(−)	(−)
Formic acid	(+) (trace)	(+) (distinct)
In the residue		
Lactic acid	(+) (distinct)	
Succinic acid	(−)	
In the water extract of the natural sample		
Tryptophan	(+)	(+)

Summary of the Results

1). Various samples, examined at different stages of ripening, gave all acid reaction chiefly due to lactic acid.

2). Autolysis is going on in the freshly prepared "Shiokara," and decreases gradually as the ripening process proceeds.

3). The enzymes found in "Shiokara" are diastase, lipase, and trypsin. The last one acts not only in weak alkaline solution but also even in neutral or in faintly acid reaction.

4). Micro-organisms play also some important rôle during the ripening process.

[1] (+), indicates presence; (−) absence.

5). Temperature has also great influence upon the action of enzymes and microbes.

6). During the ripening process, the increase of soluble matter non-albuminoid nitrogen, especially monoamino-nitrogen, and the decrease of protein, organic bases, creatine, creatinine, and purin bases were observed.

In conclusion I express my thanks to Profs. U. Suzuki and S. Machida for their kind advices given during the work.

QUANTITATIVE DETERMINATION OF CREATINE, CREATININE AND MONOAMINO-ACIDS IN SOME FISHES, MOLLUSCA AND CRUSTACEA

By Y. Okuda

College of Agriculture, Imperial University, Tokyo

I. Creatine and Creatinine

For the determination of creatine and creatinine, the flesh freed from bones, heads, fins, scales, and internal organs[1] was chopped and extracted with water at 50–55° for one hour. The residue was treated two times more in the same way. The whole extract was now boiled for a short time to remove most of the proteins by coagulation and filtered. The filtrate was evaporated under diminished pressure to a small volume and was divided into two portions. One portion of it served directly for the determination of creatinine after Folin's colorimetric method, while the other portion was previously boiled with nearly 4 per cent. sulphuric acid for two hours, to convert the creatine present into creatinine, and after removing the sulphuric acid by means of barium hydroxide, it was subjected to the determination after Folin. From the difference of these two determinations we can calculate the quantity of creatine originally present in the flesh, 1 mg creatinine being equivalent to 1.16 mg creatine. The results obtained were as follows:

[1] The case of clam was exception, as its whole body was used.

Name	In 100 parts of fresh substance			In 100 parts of dry matter	
	Water g	Creatine g	Creatinine g	Creatine g	Creatinine g
Bonito (Gymnonsarda affinis Cantor)	72.165	0.649	0.134	2.011	0.481
Tunnyfish (Thunnus schlegeli Steined)	72.402	0.497	0.064	1.800	0.232
"Katsuobushi" (Steamed and dried bonito)	14.808	0.453	0.660	0.531	0.775
Salmon (Oncorhynchus tshawytscha Walbaum)	63.300	0.560	0.067	1.525	0.182
Snapper (Pagrus major)	77.340	0.754	0.070	3.327	0.308
Carp (Cyprinus carpio L)	79.160	0.421	0.077	2.020	0.369
Shark	79.800	0.655	0.134	3.242	0.663
Lobster (Palinurus japonicus Gray)	79.920	Trace?	Trace?		
Crab (Neptunus pelagicus M-Edw)	84.500	Trace?	Trace?		
Cuttle-fish (Sepia esculenta Hoyle)	81.699	Trace	Trace		
"Kakisurume" (Chopped and dried cuttle-fish)	27.570	Trace	Trace		
Clam (Cytherea meretrix L)	90.490	Trace	Trace		

The materials used for the determination were very fresh, except the salted flesh of salmon.

We see from the above result that all of the examined fishes contained comparatively much creatine and creatinine,[1] on the contrary in mollusca and crustacea, the existence of these two compounds was doubtful, at least, they must be present only in traces. In fresh fish we found generally more creatine than creatinine, while in dried bonito the reverse was observed. It is therefore possible that a part of creatine is transformed into creatinine during the preparation of the food.

[1] Van Hoogenhuyze and H. Herploegh found per kilogramm flesh of ox, sheep, pig and horse 4.4, 4.1, 4.5 and 3.8 g creatine respectively. (Zeitschr. f. physiol. Chem., 1905, 46, 432.)

It may be mentioned here that the water extract of clam gives only slight yellowish red coloration instantly after addition of picric acid and soda after Folin, thus showing that only a trace of creatinine is present in it, but after standing for many hours at room temperature, it takes a dark red color. After some tests we found that the glycogen, originally present in the extract, is gradually acted upon by diastatic ferments of clam itself, and the sugar thus resulted may impart this red coloration. The presence of diastatic ferment in the clam is easily shown in the usual way.

II. Monoamino-acids

For the determination of monoamino-acids Sörensen's formol titration method was adopted. Of course, this method does not hold good for every monoamino-acid, but in the case of fish flesh, the quantity of the amino-acids being very little, the method of Van Slyke is not conveniently applied.

I have made some preliminary tests also, and found that the presence of organic bases, like arginine, lysine, histidine, etc., more or less interferes with the result of the formol method, so it is better to remove these bases previously. But the presence of creatine has apparently no effect upon this determination.

150 g minced fresh flesh, free from bones, heads, fins, scales and internal organs was extracted in the similar way as mentioned above and the aqueous extract was boiled and slightly acidified with acetic acid to remove coagulable proteins, filtered, neutralized and evaporated by a low pressure to a small volume, acidified with sulphuric acid and precipitated with phosphotungstic acid in the usual way. The filtrate of the phosphotungstic precipitate, after the removal of the phosphotungstic and sulphuric acid by means of barium hydroxide, was evaporated, in neutral reaction, again to a small volume and titrated according to the usual formol method. Thus the following result was obtained:

Substance	Water	N of Mono-amino-acids in g.		Remarks
		In 100g fresh flesh	In 100g dry flesh	
Carp I.....	76.609	0.022	0.094	Tunny, bonito, porgy and cuttle fish applied to the above determination were fresh. Spiny lobster, Crussian carp and Carp I were still living when they were analyzed. Carp II was analyzed standing 50 hours after death at room temperature (12°C). The increase of monoamino-acids after that time was very insignificant.
Carp II.....	76.789	0.024	0.103	
Tunny.....	73.516	0.011	0.041	
Bonito.....	69.371	0.022	0.072	
Porgy.....	76.787	0.016	0.069	
Crussian carp..	81.998	0.035	0.194	
Spiny lobster...	75.975	0.146	0.608	
Cuttle fish.....	81.671	0.089	0.485	

We see from the above results that the contents of monoamino-acids are generally very little in fish, while mollusca and crustacea contain a little more.

III. On Different Forms of Proteins in the Flesh of Fish

For this purpose, the flesh was extracted[1] with water, alcohol, NaCl, and KOH, respectively, and the quantity of total and albuminoid nitrogen in each extract was determined according to Kjeldahl's method.

[1] 10 g fresh flesh was extracted with 100 c.c. of solvent for 24 hours at 10°C.

	Flesh	Solvent	In 100 g fresh flesh		In 100 g dried flesh		Sum of each N as 100	
			Total N	Prot. N	Total N	Prot. N	Total N	Prot. N
1.	Crussian carp	H_2O	0.746	0.476	4.144	2.644	17.171	13.583
	(Carrassius aura-	0.2%KOH	2.003	1.793	11.127	9.960	46.077	51.125
	tus L)	70%Alcohol	0.386	0.174	2.144	0.966	8.899	4.961
		10% NaCl	1.212	1.064	6.732	5.910	27.881	30.339
2.	Carp	H_2O	0.479	0.326	2.182	1.485	11.129	10.326
	(Cyprinus	0.2% KOH	1.715	1.341	7.812	6.108	39.809	43.477
	carpio L)	70% Alcohol	0.492	0.240	2.241	1.093	11.420	7.602
		10% NaCl	1.622	1.250	7.388	5.694	37.649	39.605
3.	Spiny lobster	H_2O	1.600	0.736	6.659	3.063	23.808	22.816
	(Palinurus	0.2% KOH	2.138	1.212	8.898	5.044	31.813	37.572
	japonicus Gray)	70% Alcohol	0.934	0.119	3.887	0.495	13.898	3.689
		10 % NaCl	2.048	1.158	8.544	4.819	30.474	35.898
4.	Cuttle fish	H_2O	0.932	0.351	5.085	1.915	19.984	
	(Sepia esculenta	0.2% KOH	1.775	1.223	9.864	6.673	38.059	
	Hoyle)	70% Alcohol	0.602	—	3.285	—	12.908	
		10% NaCl	1.354	—	7.387	—	29.032	

The amount of proteins extracted by alkali was generally much greater than that extracted by other solvents. The proteins soluble in 10% NaCl, as globulins, were also much, water soluble proteins as proteose and albumine not much and the proteins as prolamins very little.

IV. Form of Nitrogen in Some Marine Animals

The analytical results are shown in the following table:

Summaries

1. All of the examined kinds of fish contained comparatively much creatine and creatinine, but the flesh of mollusca only trace, in the flesh of crustacea the existence of these compounds

was doubtful. The quantity of creatine was generally much more than that of creatinine, in all fresh fishes.

2. In all marine animals examined the quantity of organic base nitrogen is much more than that of monoamino-acid nitrogen, and the amount of the latter is generally very little in fish, but somewhat much in lobster and cuttle fish.

3. Most of proteins are soluble in dilute alkali solution, the proteins soluble in 10 per cent NaCl were also much, this fact must be cared on the preservation of fish.

The experiments have been made by the writer under the direction of Professor Dr. U. Suzuki, and it is my pleasant duty to thank him for his kind advices given during the progress of the work.

	Carp I. Cyprinus carpo		Carp II. Cyprinus carpo		Bonito Gymnonsarda affinis		Porgy Pagrus major		Crussian carp Carassius auratus		Tunny Thunnus schlegeli		Spiny lobster Palinurus japonicus		Cuttle-fish Sepia esculenta	
	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.
Water[1]	76.609	—	76.789	—	69.371	— 6	76.787	—	81.998	—	73.516	—	75.974	—	81.671	—
Dry matter	23.391	100.000	23.211	100.000	30.629	100.000	23.213	100.000	18.002	100.000	26.484	100.000	24.025	100.000	18.329	100.000
Total-N	2.608	11.149	2.590	11.057	4.479	14.621	3.121	13.445	2.655	14.746	3.549	13.397	3.558	14.808	2.496	13.619
Alb.-N	2.107	9.007	1.972	8.495	3.799	12.401	2.685	11.584	2.285	12.691	—	—	2.637	10.975	2.209	12.051
Non-alb-N	0.501	2.142	0.618	2.562	0.680	2.220	0.432	1.861	0.370	2.055	—	—	0.921	3.833	0.287	1.568
Warm water soluble-N.	0.884	3.779	—	—	1.628	5.314	1.025	4.419	1.039	5.771	1.530	5.776	1.586	6.601	0.919	5.013
Under that: Alb.-N	0.431	1.843	—	—	0.948	3.094	0.593	2.555	0.456	2.533	0.812	3.065	0.621	2.585	0.479	2.613
Organic base-N	0.226	0.966	0.262	1.129	0.189	0.617	0.088	1.379	0.177	0.983	0.285	1.076	0.610	2.539	0.201	1.096
Monoamino-N	0.022	0.094	0.024	0.103	0.022	0.072	0.016	0.069	0.035	0.194	0.011	0.041	0.146	0.608	0.089	0.485
Ammonium-N	Trace		Trace		Trace		Trace		Trace		Trace		Trace		Trace	
Reaction of flesh	Amphich romatic		Very faintly acid		Acid		Faintly acid		Amphich romatic		Acid		Amphich romatic		Amphich romatic	
Water[2]	79.160	—			72.165	—	77.340	—			72.402	—	79.920	—	81.699	—
Creatine	0.421	2.020			0.649	2.011	0.754	3.327			0.467	1.800	Trace?	—	Trace	—
Creatinine	0.077	0.369			0.134	0.481	0.070	0.308			0.064	0.232	Trace?	—	Trace	—
Creatine-N	0.135	0.648			0.208	0.645	0.242	0.767			0.159	0.577				
Creatinine-N	0.029	0.137			0.050	0.179	0.026	0.115			0.024	0.086				

[1] Determined September, 1910.
[2] Determined February, 1911.

THE EFFECT OF MODIFYING THE GLUTEN SURROUNDING OF FLOUR

GEO. A. OLSON

Pullman, Wash.

The results included in this article are a part of our cereal investigation work. This particular article deals with the modifying of the gluten surroundings in flour with a view of searching for the causes which affect the quality of the flour for baking purposes.

The oldest idea is that the gluten in flour holds the gas in panary fermentation. The capabilities to distend depends upon the physical qualities of gluten. Wood believes the quantity of the gluten is modified according to the amount of salts and acid present, which in turn influences the shape of the loaf, and treats this subject fully in his article on "The Chemistry of the Strength of Wheat Flour." *

The writer in the following experiments studied the effect of adding directly definite amounts of acid, alkali, or salt to original dialyzed, decanted, dough, gliadin-free and nitrogen-free with gliadin added flours. The nitrogen components of treated and untreated flour have also been included. The quantity of dry gluten and the nitrogen in the gluten of these respective flours was determined in each case.

In order to remove the salts from a flour by either dialysis or decantation, it is obvious that the flour must be soaked in distilled water and then dialyzed or allowed to stand and the supernatant liquid removed after the flour has settled to the bottom of the jars. This method at once brought up the question as to whether or not a flour could be water soaked and remilled into flour again. The first trial with dialyzed flour, however, determined that such a procedure was possible.

Perhaps the greatest difficulty experienced in this undertaking was the drying of the water-soaked flour. Aside from avoiding

* Journ. Agr. Sc. Vol. 2, No. 2, Apr. 1907, p. 139, ibid. Vol. 2, No. 3, Dec. 1907, p. 267.

too high temperatures, in the drying process, the possibilities for fermentation were most favorable and in consequence the flour while drying required the strictest attention of both an assistant and myself. The tendencies for fermentation were particularly noticeable in the flour which had been dialyzed, in spite of the fact that they had been treated frequently with small quantities of chloroform.

In addition to recovering the water-soaked flour, the dialysate and water decantations were saved and either reduced to small volume or evaporated to dryness.

The method of preparing the dialyzed, decanted, and dough flours are described in the following paragraphs:

Dialyzed Flour. Approximately kilo lots of flour were thoroly worked into paste with water and then dialyzed in a cool room, frequently changing the dialysate for a fresh supply of distilled water. Sufficient chloroform was used to check fermentation. After a course of three days the dialysis was considered complete. The colloidal material was then thoroly stirred and poured thinly over glass window panes and allowed to dry (frequently stirring) at a low temperature. When it solidified into lumpy masses all danger of fermentation was apparently removed. The lumpy masses were then worked out into thin layers and allowed to dry over night without any attention. The following morning these thin layers of flour crumbled readily and were then ground into small pieces about the size of clover seed, when it was dried further. When satisfactorily dry the work was considered complete after milling and finally bolting thru a 10xx bolting cloth.

Decanted Flour. Kilo lots of flour were worked into paste and then diluted with four liters of distilled water for every kilo of flour; after settling, the supernatant liquid was removed, and the operation frequently repeated for a period of three days, after which the flour was finally dried in the same manner as described for the dialyzed flour. Sufficient chloroform was used throughout the experiments to allay fermentation.

Dough Flour. Not knowing what influence the water would have upon the physical properties of the flour, it was necessary, in order to obtain checks, to treat the flour with enough water to make a dough, allowing it to stand for three to four hours,

then rolling this dough out into thin sheets, crumbling and milling as described for the dialyzed flour.

Plan of Investigation. After the treated flours were prepared as described above, the next step consisted in planning a method whereby the physical properties of the glutens could be studied. After some consideration and thought it was decided that in place of taking bits of gluten prepared from these flours and subjecting them to acid, alkali or salt as Wood did, that the flour be treated with these agents directly and the changes occuring, if any, be noted, and the amount of dry gluten and the nitrogen in the gluten be determined. It can readily be seen that such a plan takes into account other things besides salts and acids, since it would also determine the effect of adding these reagents to the flour directly. Later baking tests of flour treated similarly could be made and the influence of the reagents upon the shape of the loaf be noted.

Gluten Determinations. Ten gram lots of the treated and original flour were mixed with six cubic centimeters of either distilled water or N–10 normal hydrochloric acid, sulfuric acid, phosphoric acid, sodium hydroxide, potassium hydroxide, dipotassium acid phosphate, disodium acid phosphate, dicalcium acid phosphate, sodium chloride, sodium sulfate, aluminum sulfate and magnesium sulfate, and worked up into small wads. These wads of flour were then allowed to stand for one hour, after which they were finally washed over silk with running water. In some cases the particles of gluten cohered and were easy to gather, while in other instances the gluten particles scattered and fell upon the silk. All scattering glutens which fell upon the silk, together with those that cohered, were gathered together, washed, dried and weighed according to the usual method. The length of time required for drying was 20 hours at maximum temperature of the water oven. Nitrogen determinations of the dry glutens were finally made by the Kjeldahl method. The results of the percent of gluten, weight and percent of nitrogen in the gluten, percent of total nitrogen and percent of total nitrogen calculated from the nitrogen in the gluten from the original flour as 100, are given in the following table, which is subdivided into four separate parts according to the treatment of the flour.

TABLE I (a, b, c and d)

EFFECT OF MIXING ACID, SALT OR ALKALI WITH DIFFERENTLY PREPARED FLOURS UPON THE YIELD OF AND NITROGEN IN GLUTEN

		H_2O	H_2SO_4 N/10	H_3PO_4 N/10	HCl N/10	NaOH N/10	KOH N/10	Na_2HPO_4 N/10	K_2HPO_4 N/10	$CaHPO_4$ N/10	NaCl N/10	Na_2SO_4 N/10	$MgSO_4$ N/10	$Al_2(SO_4)_3$ N/10
(a) *Original*	Per Cent Gluten	11.94	7.73	6.43	3.56	9.30	8.84	12.07	10.85	10.89	12.82	11.59	11.70	10.39
	Weight of Nitrogen	0.1521	0.101	0.079	0.043	0.121	0.120	0.149	0.142	0.141	0.149	0.155	0.146	0.139
	Per Cent Nitrogen	12.75	13.08	12.22	12.09	13.02	13.58	12.34	13.10	12.97	11.60	13.41	12.46	13.42
	Per Cent of total Nitrogen	93.30	61.97	48.15	26.35	74.25	73.56	91.33	87.12	86.52	91.07	95.34	89.50	85.55
	Per Cent Gluten Nitrogen in Original Flour	100.00	66.42	51.61	28.34	79.58	78.84	97.89	93.38	92.73	97.61	102.18	95.93	91.69
(b) *Dough*	Per Cent Gluten	11.51	5.84	9.20	1.48	9.47	9.18	11.75	10.92	10.80	11.21	.....	.....	.....
	Weight of Nitrogen	0.152	0.074	0.125	0.020	0.116	0.126	0.151	0.150	0.151	0.151	.....	.....	.....
	Per Cent Nitrogen	13.19	12.33	13.54	13.54	12.20	13.25	12.88	13.66	13.95	13.49	.....	.....	.....
	Per Cent of total Nitrogen	93.05	45.32	76.37	12.27	70.94	77.34	92.79	91.42	92.36	92.70	.....	.....	.....
	Per Cent Gluten Nitrogen in Original Flour	99.72	48.58	81.85	13.16	76.03	83.54	99.45	97.98	98.99	99.36	.....	.....	.....
(c) *Dialyzed*	Per Cent Gluten	10.47	0.951	3.02	0.381	9.48	9.21	11.70	10.41	9.91	10.69	11.04	11.03	7.13
	Weight of Nitrogen	0.147	0.013	0.040	0.005	0.133	0.124	0.156	0.144	0.136	0.152	0.152	0.152	0.094
	Per Cent Nitrogen	14.00	13.70	13.38	13.23	14.02	13.48	13.36	13.84	13.72	14.18	13.97	13.81	13.11
	Per Cent of total Nitrogen	86.53	7.79	23.80	2.97	78.51	73.31	92.23	85.04	80.25	89.50	90.00	90.13	55.34
	Per Cent Gluten Nitrogen in Original Flour	96.32	8.67	26.49	3.31	87.39	81.60	102.67	94.66	89.33	99.63	99.90 $Ca(OH)_2$	100.09 $C_2H_5(OH)_2$	61.45 C_2H_5OH
(d) *Decanted*	Per Cent Gluten	0.0041	0.004	0.000	0.000	6.88	6.98	0.000	0.000	0.000	0.008	5.46	0.59	0.000
	Weight of Nitrogen	.....	.....	.....	.....	0.0998	0.0948	.....	.....	.....	.....	0.0713	0.008	.....
	Per Cent Nitrogen	.....	.....	.....	.....	14.55	13.58	.....	.....	.....	.....	13.05	12.80	.....
	Per Cent of total Nitrogen	.....	.....	.....	.....	60.12	57.10	.....	.....	.....	.....	43.92	4.55	.....
	Per Cent Gluten Nitrogen in Original Flour	.....	.....	.....	.....	65.58	62.28	.....	.....	.....	.....	46.82	4.93	.....

Comparing the data obtained for dry gluten in the original dough, dialyzed, and decanted flours as modified by either water, acid, alkali, or salt, it will be noted that with the exception of sodium sulfate none of the N/10 solutions of salt, acid, or alkali gave as high results as was obtained with the water in the original flour. In Table 1 (a) the prejudicial* influence upon the gluten increased in the order named, disodium acid phosphate, sodium chloride, magnesium sulphate, dipotassium acid phosphate, dicalcium acid phosphate, aluminum sulfate, sodium hydroxide, potassium hydroxide, sulfuric acid, phosphoric acid, and hydrochloric acid.

It will be further noted from the results given in table 1 (b) that the dough flour agrees fairly well with the original flour, the widest variations occurring where the flour had been treated with sulfuric acid, phosphoric acid, and hydrochloric acid. In the same way the weight of nitrogen varied and the percent of total nitrogen calculated on the basis of 100 for the original flour perhaps illustrates more clearly the effect of the acid upon the dough flour when compared with the original.

In regard to the dialyzed flour, when compared with the original, water has affected the yield of gluten and the weight of nitrogen to a slight extent. Sulfuric acid, phosphoric acid and hydrochloric acid have been prejudicial even to a much greater extent than was the case in the dough flour. Sodium hydroxide and potassium hydrocide were apparently beneficial,** the former more so than the latter. Comparing salts of phosphoric acid, the sodium salt increased the gluten and nitrogen content to a greater extent than the potassium salt, while the calcium salt was slightly prejudicial in this case, being an exception to that observed in case of the dough flour. Sodium chloride was beneficial when weight of the nitrogen contained in the gluten is considered. On the basis of weight of nitrogen, aluminum sulfate was prejudicial, sodium sulfate was without effect and magnesium sulfate was beneficial. Comparing the influence of

* Prejudicial refers to decreased amounts of dry gluten or nitrogen in the gluten.

** Beneficial refers to an increased amount of dry gluten or nitrogen in the gluten.

the reagents on the gluten content and weight of nitrogen with water, it will be noted that disodium acid phosphate, magnesium sulfate, sodium sulfate and sodium chloride were beneficial in the order named, while dipotassium acid phosphate, dicalcium acid phosphate, sodium hydroxide, potassium hydroxide, aluminum sulfate, phosphoric acid, sulfuric acid and hydrochloric acid were prejudicial in the order named.

The dalysate from dialyzed flour (based upon the weight of flour used) contained 0.655 percent total solids, of which 0.57 per cent was combustible and 0.084 percent was ash.

The flour obtained after decanting the soluble extract gave the most remarkable results of any in the series. Neither water, salts, nor acid yielded gluten, while sodium and potassium hydroxides were beneficial to gluten formation. In addition, calcium hydroxide, glycerol, alcohol and flour extract were tried and only the glycerol and calcium hydroxide were found to be beneficial.

Collectively, the results given in Table I (a, b, c and d) clearly show that acid is more prejudicial than alkali of same normal strength and the salts with one exception (aluminum sulfate in case of the dialyzed flour) has practically a very slight effect upon the physical properties of the gluten. On this basis, sodium was least active and calcium most active on gluten disintegration. There is no doubt but that the substances contained in the water extract play an important part in modifying the physical properties of the gluten. Whether the substances contained in this extract, which play so important a role, are inorganic or not must be determined. It appears that the (OH) radical tends to produce coherence. This view is supported from the results obtained in the dialyzed flour when compared with the original, and in the decanted flour where other substances failed to bring on coherence. Just what causes the disintegration of the gluten complex has not as yet been satisfactorily established.

Physical Condition of the Gluten. The gluten in the flour studied from physical appearances was fair. None of the salts used showed any marked variation in the physical appearances of the gluten other than was observed in the case of water. N/10 acids tended to produce scattering glutens, which when

TABLE II

EFFECT OF MIXING ACID, SALT OR ALKALI WITH PATENT FLOURS FROM DIFFERENT LOCALITIES UPON THE YIELD AND NITROGEN IN GLUTEN

	HCl N/100	HCl N/10	HCl N/1	H_2SO_4 N/100	H_2SO_4 N/10	H_2SO_4 N/1	$C_3H_6O_3$ N/100	$C_3H_6O_3$ N/10	$C_3H_6O_3$ N/1	NaOH N/100	NaOH N/10	Na_2HPO_4 N/10	NaCl N/10	Na_2SO_4 N/10	H_2O
Pullman Flour.															
Per Cent dry Gluten ..	7.85	0.94	trace	7.45	1.90	3.11	7.43	3.17	none	7.77	4.96	8.33	8.06	8.04	8.01
" Nitrogen ..	12.43	10.85	.....	13.04	13.03	10.52	12.90	. . ....	.....	11.90	12.15	12.40	12.68	12.78	12.32
" of total Nitrogen.....	82.70	8.66	.....	82.00	21.03	27.77	81.15	.....	.....	78.30	51.02	87.56	86.61	87.09	83.64
Bridgeport Flour.															
Per Cent dry Gluten .	12.56	2.55	0.07	12.40	4.44	4.53	11.74	0.98	0.11	14.38	7.96	12.51	11.85	12.13	12.09
" Nitrogen	12.00	12.86	... .	12.09	13.77	11.93	12.10	13.47	.....	10.84	14.30	12.73	12.70	13.02	12.62
" of total Nitrogen.....	84.26	18.30	.....	83.69	34.10	30.19	79.39	73.58	.....	87.10	63.59	88.93	84.00	88.22	85.25
Vancouver Flour.															
Per Cent dry Gluten ..	10.90	5.36	0.08	9.53	6.68	6.45	.....	.....	.....	12.43	9.30	12.07	12.82	11.59	11.94
" Nitrogen. . ..	12.54	12.04	.....	13.89	13.83	10.77	.....	.....	.....	11.50	13.02	12.34	11.60	13.41	12.75
" of total Nitrogen.....	83.83	39.56	.....	81.25	56.69	42.58	.....	.....	.....	89.57	74.30	91.39	91.13	95.33	93.36
Anatone Flour.															
Per Cent dry Gluten ..	8.52	2.48	0.000	9.06	4.22	0.00	8.33	0.000	0.000	.....	4.11	8.53	8.45	8.47	8.18
" Nitrogen	13.63	13.88	.....	13.06	12.75	.....	13.28	.....	.....	.....	13.90	13.00	14.17	13.93	14.28
" of total Nitrogen.....	83.00	24.57	.....	84.50	41.07	.....	79.07	.....	. .....	.....	40.79	79.21	85.50	84.28	83.50

It is difficult to obtain gluten from flour treated with N/1 sodium hydroxide.

gathered were dry and seemingly free from water, while alkalies of the same strength affected the gluten in such a way that in the process of washing out the starch the gluten rolled or washed down with the water on to the silk in little bits, which when collected together, (the gluten) had a very dry touch, indicating that glutens treated in this way contain very little water. The amount of water contained in N/10 acid and alkali prepared glutens was not determined.

Influence of Acids, Alkalies and Salts on Gluten from Patent Flours. Four patent flours were next studied with the object of determining whether or not the addition of acid, alkali, or salt to the flour modified the physical properties, the yield and the nitrogen content of the gluten in these flours. The flour selected for this series of investigations represent four different localities with different climates, soil and types of wheat. The glutens from these flours varied considerably in their physical qualities.

As in the case of the previous experiments, ten gram samples were mixed with six cubic centimeters of either N/10 acid, alkali or salt, and in addition to these like qualities of N/100 and N/1 acid and alkali were tried. These different lots were worked up into wads and the gluten collected by washing over silk. The weight and the per cent of the nitrogen in the gluten are recorded in Table II. The grams of nitrogen in the gluten and the ratio of total nitrogen in the flour to nitrogen in the gluten are recorded in Table III. In place of phosphoric acid, lactic acid was used in these experiments. Potassium hydroxide was not tried.

It will be noted from the data given in Table II that the acids, alkali and salt affected the weight of gluten similarly as found in Table I (a). While the strength of N/100 acid was slightly prejudicial, increasing the strength to N/10 and N/1 had marked effects upon the yield of gluten when compared to those treated with distilled water and run as controls. The increased or equal weight of gluten in the flour treated with N/1 sulfuric acid compared to N/10 sulfuric acid was due to the fact that the former was poorer in nitrogen. Disodium acid phosphate was beneficial in all cases. Sodium chloride and sodium sulfate were beneficial in three out of four instances. It was impossible

TABLE III

GRAMS OF NITROGEN RECOVERED IN THE GLUTEN AND THE RATIO OF TOTAL NITROGEN TO GLUTEN NITROGEN FROM PATENT FLOURS TREATED WITH ACID, SALT, OR ALKALI

	HCl N/100	HCl N/10	HCl N/1	H_2SO_4 N/100	H_2SO_4 N/10	H_2SO_4 N/1	$C_3H_6O_3$ N/100	$C_3H_6O_3$ N/10	$C_3H_6O_3$ N/1	NaOH N/100	NaOH N/10	$NaHPO_4$ N/10	NaCl N/10	Na_2SO_4 N/10	H_2O
Pullman Flour.															
Grams N. in Gluten....	0.0976	0.0102	None	0.0967	0.0248	0.0328	0.0958	None	None	0.0924	0.0602	0.1033	0.1022	0.1028	0.0987
Ratio of total to Gluten Nitrogen	1:0.827	1:0.087	.....	1:0.820	1:0.210	1:0.278	1:0.811	.....	.....	1:0.783	1:0.510	1:0.876	1:0.866	1:0.871	1:837
Bridgeport Flour.															
Grams N. in Gluten...	0.1508	0.0328	None	0.1498	0.0610	0.0540	0.1421	0.0132	None	0.1558	0.1138	0.1592	0.1504	0.1579	0.1526
Ratio of total to Gluten Nitrogen.....	1:0.843	1:0.183	.....	1:0.837	1:0.341	1:0.302	1:0.794	1:0.736	.....	1:0.871	1:0.636	1:0.889	1:0.840	1:0.882	1:0.853
Vancouver Flour.															
Grams N. in Gluten ...	0.1366	0.0645	None	0.1324	0.0924	0.0694	.....	.....	.....	0.1460	0.1211	0.1490	0.1485	0.1554	0.1522
Ratio of total to Gluten Nitrogen.....	1:0.838	1:0.396	.....	1:0.813	1:0.567	1:0.426	.....	.....	.....	1:0.896	1:0.743	1:0.914	1:0.911	1:0.953	1:0.934
Anatone Flour.															
Grams N. in Gluten ...	0.1162	0.0344	None	0.1183	0.0575	None	0.1107	None	None	.....	0.0571	0.1109	0.1197	0.1180	0.1169
Ratio of total to Gluten Nitrogen.....	1:0.830	1:02.46	.....	1:0.845	1:0.411	.....	1:0.791	.....	.....	.....	1:0.408	1:0.792	1:0.855	1:0.843	1:0.835

to obtain gluten from flour treated with N/1 sodium hydroxide. N/100 sodium hydroxide was less prejudicial than N/10. The most peculiar modification of gluten that took place was with N/100 sodium hydroxide in case of the Bridgeport bluestem where the weight of the gluten increased. The per cents of nitrogen in the glutens varied irregularly.

The data given in Table III show the actual amounts of nitrogen entering into the gluten make-up. The ratio of total nitrogen in the flour to that found in the gluten shows how reagents of identical normal strengths modify the nitrogen complexes in the gluten. Particular attention should be called to the effect of N/100 acid. The ratio constant was remarkably uniform for each regardless of source or nature of the flour. Slightly higher ratios were obtained with hydrochloric acid and sulphuric acid than with lactic acid. With N/10 solution, sulphuric acid was the least prejudicial. Hydrochloric acid disintegrated the gluten rapidly at this strength, while with lactic acid practically no gluten was obtained. No results could be obtained with either N/1 hydrochloric acid or N/1 lactic acid. The accompanying cut illustrated the effect of N/100, N/10 and N/1 sulphuric and hydrochloric acids upon the yield of gluten.

(CUT) (1) (See legend on back of photo.)

The physical condition of the glutens resulting from washing the flour after it had been treated with hydrochloric acid, lactic acid, and sodium hydroxide of N/100, N/10 and N/1 strengths are tabulated in Table IV.

From the physical appearances of the gluten as affected by either acid or alkali, it may be said that alkali behaves similarly to acid of the same normal strength.

Flour with Gliadin Removed. Since it is more difficult to remove glutenin than gliadin from flour it was decided to study the influence of acid, alkali, and salts on flour with the gliadin removed. Flour was repeatedly extracted with cold 70 per cent alcohol and finally dried, milled and bolted thru a 10xx bolting cloth. As in previous experiments, 10 grams of flour and 6 cubic centimeters of water or specified reagent were used. The amount of gluten obtained with water 0.14%, N/10 sodium

TABLE IV

Physical Properties of Gluten as Affected by Acid and Sodium Hydroxide

	HCl	H_2SO_4	$C_3H_6O_3$	Na (OH)
N/1	disintegrated	soft, massive, lack cohesiveness.	disintegrated	tough dough, soapy and difficult to remove gluten
N/10	separated in particles, firm and seemingly dry	same as for N/10 HCl	disintegrated	separates into particles, firm and dry
N/100	excellent cohesion, elastic	same as for N/100 HCl	same as for N/100 HCl	soft, voluminous gluten, easy to wash, elastic

hydroxide 0.712%, N/10 sodium chloride 0.100% and N/10 hydrochloric acid none, was insignificant when compared with flours treated by dialysis or decantation and as a result it was considered futile to try other reagents on this gliadin-free flour. These results, however, appear to indicate that gliadin does not act as an acid, alkali, or salt, for if such was the case, it is reasonable to believe that all of the glutenin could have been recovered by the addition of an acid, alkali, or salt.

Nitrogen-free Flour with Gliadin Added. In the previous paragraph, the effect of acid, alkali, and salt upon gliadin-free flour was discussed. In a similar way the same original flour was treated with weak potassium hydroxide N/50 several times. The supernatant liquid resulting after the flour stood for four hour intervals was replaced with fresh lots of potassium hydroxide and finally the treated flour was washed with frequent changes of distilled water, then rolled into thin layers, dried and milled. The amount of nitrogen components present after the above treatment was determined and found to be a trace of globulin and albumin nitrogen, amid nitrogen 0.119%, alcohol soluble (gliadin) nitrogen 0.133%, total nitrogen 0.301%. The total nitrogen in the original flour was 1.63%.

It appears from these results that water is more prejudicial than either N/100 hydrochloric acid or sodium hydroxide and N/10 hydrochloric acid or sodium chloride. It is reasonable to believe, however, that such was not the case, since the gliadin in the course of preparation and purifying has been altered and when dried and ground is made up of minute hard particles of gliadin. Just as dried gluten takes up water only after long periods of soaking, so it is with these hard particles of gliadin which, when mixed with water, require long periods of time to even swell. Thus when flour is incorporated with an addition of these hard particles of gliadin and then mixed into wads with water and gluten determinations made, the finest particles of gliadin pass thru the fine silk and are lost. The only gliadin that can be recovered are the partly swelled particles which are too large to pass thru the silk. The presence of N/100 hydrochloric acid or sodium hydroxide and N/10 hydrochloric acid and sodium chloride, undoubtedly, has increased the tendencies of the hard particles of gliadin to absorb more water than was the case with distilled water and as a result increased the number of particles too large to pass thru the silk hence a larger yield. With larger quantities of water than was used in these experiments more gliadin would undoubtedly have resulted since gliadin takes up water in time and with an increased amount accelerates water absorption thereby increasing the number of larger particles.

Nitrogen Components of Treated and Untreated Flour. Aside from studying the effect of acid, alkali and salts upon the yield of gluten and the weight of nitrogen in the same, a systematic study of the nitrogenous components in the flours mentioned above was made. The first flours investigated were the dough, dialyzed, and decanted compared with the original. All nitrogen determinations were made according to the straight Kjeldahl method. The total nitrogen, the percent of nitrogen of flour entering into the gluten makeup, the percent of alcohol soluble nitrogen (using 70% alcohol by volume), the percent of nitrogen compounds precipitated by phosphotungstic acid from a one percent sodium chloride soluble of flour and the percent of nitrogen in the salt soluble extract not precipitated by phosphotungstic acid were determined.

TABLE V

Nitrogen Components in Differently Treated Flours

	Nitrogen in Flour Per Cent.	Nitrogen in Gluten Per Cent.	Gliadin Nitrogen Per Cent.	Edestin and Leucosin Nitrogen Per Cent.	Amide Nitrogen Per Cent.	Glutenin Nitrogen based on Flour Per Cent.	Glutenin Nitrogen based on Gluten Per Cent.
Original	1.63	1.5218	0.602	0.378	0.056	0.594	0.4858
Dough	1.63	1.5176	0.609	.....	.....	.....	.....
Dialyzed	1.69	1.4658	0.623	0.308	0.070	0.689	0.4648
Decanted	1.66	1.4255*	0.756	0.273	0.077	0.554	0.3195

The results given in Table V show a higher nitrogen content for the dialyzed and decanted flours, than was found in case of dough and original flour. These differences in total nitrogen in the dialyzed and decanted flours may be due to the removal of nitrogen-free or nitrogen-poor material. The fact that the decanted flour contains less nitrogen than the dialyzed flour would tend to indicate that this was not the case; on the other hand, it must be considered that small quantities of the more highly complex nitrogen bodies are carried away with water and with the large quantities of water used repeatedly for decantation some of the higher nitrogen bodies have been affected and their losses have resulted in diminishing the nitrogen content. The amount of nitrogen in the glutens (expressed in percent of the nitrogen of the flour in gluten), also supports this thought, being higher in amounts for the original and dough than in either the dialyzed or decanted. Although, it was impossible to obtain any gluten with water (see Table 1, d) in the decanted flour, the result 1.4295% nitrogen cannot be far off from the actual amount present if a gluten could have been made. This result has been calculated from the data given in table 1, a & d, and confirmed by those given in Table V. The increased amount of alcohol soluble nitrogen in the decanted flour cannot be entirely accounted for by the removal of the nitrogen-free and nitrogen-poor material. In this same flour there is less precipitated nitrogen and more nitrogen not precipitated by phosphotungstic acid. The same is true to a less

* Calculated.

extent in case of the dialyzed flour. The glutenin was obtained by difference between the nitrogen obtained in gluten and the sum of alcohol and the salt soluble. Another column for glutenin has been inserted for the benefit of those who subtract the alcohol and salt soluble from the total nitrogen.

In Table VI the results given in Table V are calculated as proteins using the factor 6.25.

TABLE VI

Nitrogen Components Calculated as Proteins

	Protein in Flour Per Cent.	Gliadin Per Cent.	Edestin and Leucosin Per Cent.	Amides Per Cent.	Glutenin based on Flour Per Cent.	Glutenin based on Gluten Per Cent.	Gluten Per Cent.
Original	10.19	3.76	2.36	0.36	3.71	3.04	9.51
Dough	10.19	3.81	....	....	....	....	9.49
Dialyzed	10.56	3.89	1.93	0.43	4.31	2.91	9.16
Decanted	10.38	4.73	1.70	0.49	3.46	2.00	8.91

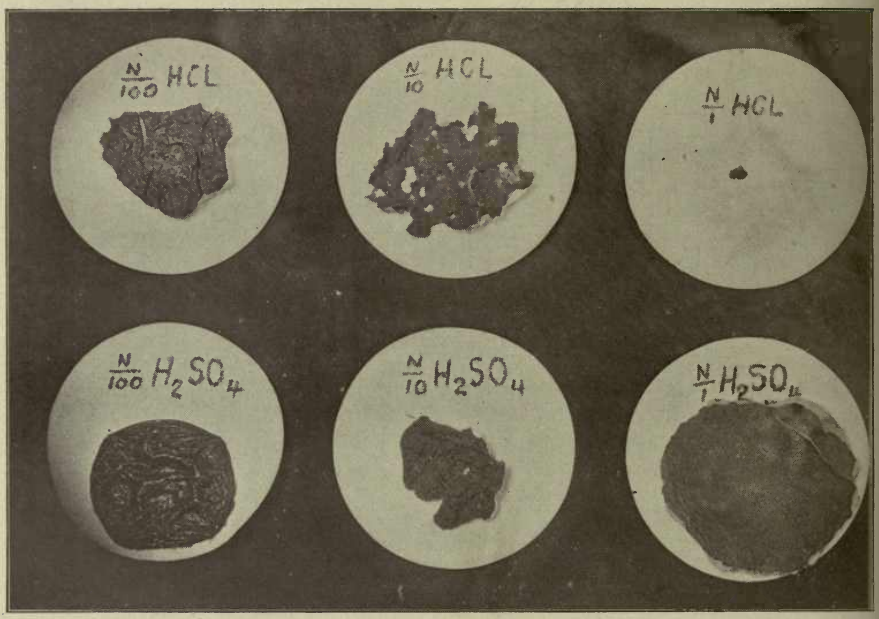
Owing to the increased amount of alcohol-soluble nitrogen obtained in the decanted flour over that found in the original, two other flours were treated by the decantation method. The nitrogen components of these flours in conjunction with the same flours untreated were also made. The data for the original and decanted flour given in Table V are included in Table VII for comparison.

TABLE VII

Nitrogen Components in Patent Flour Compared with the Same Flour after Decantation.

	Total Nitrogen Per Cent.	Gluten Nitrogen Per Cent.	Gliadin Nitrogen Per Cent.	Edestin and Leucosin Nitrogen Per Cent.	Amide Nitrogen Per Cent.	Glutenin Nitrogen based on Flour Per Cent.	Glutenin Nitrogen based on Gluten Per Cent.
Pullman, Original	1.18	0.987	0.651	0.329	0.028	0.172	None
Decanted	1.20	.....	0.686	0.217	0.021	0.256	.....
Bridgeport, Original	1.79	1.526	0.910	0.350	0.049	0.481	0.217
Decanted	1.74	.....	0.950	0.245	0.035	0.510	.....
Vancouver, Original	1.63	1.5218	0.602	0.378	0.056	0.594	0.486
Decanted	1.66	1.4255*	0.756	0.273	0.077	0.554	.....

* Calculated.



Increasing the strength of acid alters the physical quality of gluten.

It will be seen from the results given in Table VII that the amount of alcohol-soluble nitrogen in the decanted flours were again found to be higher than that found in the original flours. The amount of amid nitrogen found was, however, the reverse of that found in Table V. When the nitrogen in the gluten makeup is assumed to be composed of the gliadin and glutenin it will be noted that the Pullman flour is devoid of glutenin. On the other hand, if the total nitrogen is considered instead of the nitrogen entering into the gluten makeup there is found 0.172% of gluten.

Extract from Decanted Flour. In addition to having determined the nitrogen components of both the control and the decanted flours, the extract of the decanted flour was also studied in regard to the total nitrogen, nitrogen precipitated and not precipitated by phosphotungstic acid. The total solids and total ash were also made. These results are recorded in Table VIII.

TABLE VIII

Nitrogen Components in Decanted Liquid from Patent Flour

	Total Nitrogen Percent.	Edestin and Leucosin Nitrogen Percent.	Amide Nitrogen Percent.	Total Ash Percent.	Total Solids Percent.
Pullman.....	0.094	0.079	0.015	0.36	3.00
Bridgeport.....	0.081	0.074	0.007	0.38	2.74

The results given in Table VIII show that the total solids and nitrogen components for the Bridgeport flour are slightly lower than was the case in the Pullman flour. It may be of interest to know what these extracted substances are, since all or some one of them may be important in determining the baking qualities of a flour. Although some work has been done by the writer along this line of research, nothing definite has been determined thus far.

In Table IX an attempt has been made to correlate some of the data given in preceding tables. The ratio of total nitrogen to gluten nitrogen, as affected by water, sodium hydroxide, disodium

acid phosphate, and sodium sulphate on the one hand, with the ratio of gliadin to glutenin nitrogen on the other, are recorded.

TABLE IX

Ratio of Total Nitrogen to Gluten Nitrogen as Affected by Alkali, Water and Salt and Ratio of Gliadin to Glutenin Nitrogen

	Ratio of gliadin to-gluten nitrogen	Total nitrogen to gluten nitrogen ratio			
		N/10 Na (OH)	Water	N/10 $Na_4H_2(PO_4)_2$	N/10 $Na_2(SO_4)$
Pullman	1:0.264	1:0.57	1:0.837	1:0.87	1:0.87
Bridgeport	1:0.528	1:0.636	1:0.852	1:0.88	1:0.88
Vancouver	1:0.986	1:0.743	1:0.934	1:0.91	1:0.95

When flour was treated with either water, alkali or salt the ratio of total nitrogen to gluten nitrogen decreased as the ratio of gliadin nitrogen to glutenin nitrogen decreased; this fact is clearly brought out in table IX. In other words, the more glutenin nitrogen a flour contains the higher will the gluten nitrogen be. On the other hand, this view is contradicted, since the flour which had the gliadin removed practically yielded no gluten when treated with acid, alkali, or salt. In the same way decanted flour having nearly all of its glutenin and gliadin yielded only a part of its gluten when treated with sodium hydroxide, potassium hydroxide, calcium hydroxide, and glycerol. From these observations there appears to be some as yet unknown substance or physical change which is more important in causing a transformation of the physical properties of gluten than either acid, alkali, or salt. Whether this be in the form of some organic salt, acid, or alkali, or not is a problem for the future.

Conclusions

1. Flours were either made into dough with water, dialyzed or decanted, then dried, remilled and bolted into flour again. Gluten determinations were made, using different reagents in order to note the differences in yield caused by modifying the surroundings.

2. Mixing untreated flour with N/10 solutions of different salts, acids and alkali was prejudicial to the yield of gluten. The prejudicial influence increased in the following order: Sodium phosphate, sodium chloride, magnesium sulphate, potassium phosphate, calcium phosphate, aluminum sulphate, sodium hydroxide, potassium hydroxide, sulphuric acid, phosphoric acid and hydrochloric acid.

3. Flour treated with sufficient water to form a dough then dried and remilled into flour again, has resulted in slightly modifying the gluten of that flour when compared with the original. N/10 solutions of sulphuric acid, phosphoric acid and hydrochloric acid were more prejudicial to gluten formation in dough flour than in the original one.

4. The gluten from flour which has been dialyzed has been affected to a greater extent than was the case with the dough flour.

5. No gluten could be obtained from the residual flour by decantation when mixed with either water, salts or acids. Mixed with N/10 solutions of sodium, potassium, and calcium hydroxides and glycerol, varying amounts of gluten were obtained. The amount of gluten obtained decreased with the hydroxides used in the order mentioned above in No. 2 of conclusions.

6. N/10 solutions of salts appear to have no effect upon the physical appearance of the resulting gluten when compared with similar ones which were mixed with water. N/10 acid and alkali tend to produce scattering glutens which when gathered appear to be rather free from water. The amount of water held by such glutens was not determined.

7. Using patent flours from various sources it was found that the glutens prepared from these behaved similarly to those previously obtained and treated in like manner.

8. Patent flours were treated with N/100, N/10, and N/1 sulphuric acid, hydrochloric acid, lactic acid and sodium hydroxide. N/100 strength solutions had the least effect upon the yield of gluten. N/10 strength solutions, the hydrochloric acid yielded some and the lactic acid practically no gluten. Using N/1 strength solutions non-cohering gluten resulted with sulphuric acid and no yield with hydrochloric acid and lactic acid.

9. In determining the nitrogen present in the glutens resulting from treating flour with N/100 strength, solutions of sulphuric acid, hydrochloric acid or lactic acid, gave fairly concordant results when compared with one another and the ratio of total nitrogen to gluten nitrogen was practically constant, regardless of the kind of acid used.

10. Flour with the gliadin removed does not form gluten either in the presence of water, acid, alkali or salt. This fact indicates that gliadin does not behave either as an acid, alkali or salt.

11. Nitrogen free flour with gliadin added to it does not form gluten either in the presence of acid, alkali or salt. The failure to recover the admixed, previously dried, gliadin is undoubtedly due to the limited time the gliadin was exposed to water; in addition to the small quantity of water used.

12. In studying the nitrogen components of the original dough, dialyzed, and decanted flours, it was found that the dialyzed and decanted flours showed slightly higher total nitrogen and alcohol soluble nitrogen contents than was found in either the original or dough flour.

13. An attempt was made to correlate the ratio of gliadin nitrogen to glutenin nitrogen with the ratio of total nitrogen to gluten nitrogen as affected by either water, sodium hydroxide, sodium phosphate and sodium sulphate. According to the data obtained it appears that the ratio of total nitrogen to gluten nitrogen decreased as the ratio of gliadin nitrogen to glutenin nitrogen decreased. On the other hand, it must be considered that gliadin-free flour yielded no gluten and decanted flour yielded only a part of its gluten. Accordingly there appears to be as yet some unknown substance which is important in causing a transformation of the physical properties of the gluten.

A METHOD FOR THE DETECTION OF COLOR IN TEA

By E. Alberta Read, Ph.D., M.D.

Bureau of Chemistry, Department of Agriculture, Washington, D. C.

The following method was devised for the purpose of detecting color on tea, the United States Treasury Department having issued regulations prohibiting the entry into this country of colored teas.

For the demonstration of color and facing on tea, the chemical methods, as suggested by Allen (1), Leach (2), Villiers et Collin (3), and The International Committee (4), have usually been employed. The difficulty with such methods is in the small amount of color used and the masking of the color reactions by the solution of natural color in the tea.

The method suggested in this paper has the advantage in that it can detect much smaller amounts than can be found by chemical methods, but at the same time overlooks traces of color which would be found by a compound microscope.

Hilger and Mayrhofer (5) suggest a method of rubbing wet tea leaves on white paper, to detect artificial color.

The method suggested in this paper has an advantage over the use of wet tea leaves in that a larger sample can be used in a single examination, and it is more easily and quickly handled. In cases where there is a blending of colored and uncolored tea, many of the wet leaves might be used without detecting the color. These authors also suggest the sifting of the tea, but no

(1) Commercial Organic Analysis, 1911; V, 658.

(2) Food Inspection and Analysis, 1911; 375.

(3) Traité des Altérations et Falsifications des Substances Alimentaires, 1900; 258.

(4) Report on the Unification of Analytical Methods for Food-products; 1912; 148.

(5) Vereinbarungen zur Einheitlichen Untersuchung und Beurtheilung von Nahrungs-und Genussmitteln fur das Deutsche Reich, 1–3, 1897–1902; 54.

mention is made in the article of crushing the particles on paper to demonstrate the presence of color, and thus not only affording a method surer and safer for the chemist but also one which can be used by men untrained in Science; therefore, making it available for tea examiners at the ports and for the tea tasters employed by the importers and dealers in tea. It also allows the accurate handling of large numbers of samples within a short time, thus preventing the detention of tea at the ports for any considerable time and consequent financial loss to the importer.

The articles needed for testing the tea are sieves, 16 to 24 meshes to the centimeter, a spatula or case knife and a piece of unglazed, white paper.

A small amount of tea, about 25 to 50 grams, is placed in a sieve and shaken over a piece of white paper. If the tea is tightly rolled, it should be slightly crushed, either before putting into the sieve or by rubbing it against the sieve. The dust on the paper is then crushed by dragging over it a spatula or case knife, pressure being applied by the finger to the end of the spatula. This crushes not only the tea dust, but any particles of color which are present. The process of dragging the knife across the paper, streaks the color, making it more easily seen. A lens with a magnification of 8 to 12 diameters is useful in detecting the smaller streaks. Sunlight is desirable; bright light is essential for this work.

This method will detect any coloring as blue, turmeric or carbon. An application of the method has been made by Mr. G. F. Mitchell, Supervising Tea Examiner, Treasury Department, to the detection of facing on tea. Black, unglazed paper is used in place of the white paper. The facing leaves a white streak on the black paper.

Microchemical Tests for Color:

A black streak would suggest carbon; the blue may be Prussian blue, indigo or ultramarine; and a yellow streak suggests turmeric. These may be identified as follows: The carbon, by its glossy appearance; the blue and turmeric can be tested directly on the paper or by mounting on a microscopic slide. To the blue streak on the paper or to the particle on the microscopic slide, add a drop of 40 % sodium hydroxide. Prussian blue will turn

yellowish-brown; indigo or ultramarine will remain unchanged in color. Ultramarine is discolored by acid; indigo remains unchanged when treated with either acid or alkali. Turmeric turns bright red when a drop of a mixture of equal parts of boracic acid and concentrated hydrochloric are added to the yellow streak. Concentrated sulphuric also turns turmeric bright red.

RECHERCHE DE PETITES QUANTITES DE GRAISSE DE COCO DANS LE BEURRE DE VACHE

Par M. Lucien Robin
Chimiste au Laboratoire Municipal de Paris, Paris, France

La méthode d'analyse du beurre que j'expose dans un premier mémoire, est assez sensible pour déceler 10% de coco. En y apportant quelques variantes, on pourrait en augmenter encore la sensibilité, ainsi que j'ai pu le vérifier déjà par un certain nombre d'expériences que je tiens à renouveler encore, et dont les résultats me semblent assez intéressants pour que je les fasse connaître.

Le beurre de coco renfermant beaucoup d'acides caprique, caproïque et laurique dont la majeure partie se trouve dans le groupe des acides gras insolubles dans l'eau, et ces acides ayant un indice de saponification élevé, j'avais pensé qu'en isolant assez d'acides gras de ce groupe pour en déterminer cet indice, je trouverais peut-être là un renseignement précieux.

Un certain nombre de tentatives de ce genre m'ont bien fait voir que l'indice de saponification des acides de ce groupe obtenus avec le coco, était relativement élevé, et le plus souvent supérieur à celui que donnent les mêmes acides retirés du beurre pur, mais que pourtant on ne pouvait espérer pouvoir s'en tenir exclusivement à cette détermination pour affirmer la présence de 5% de coco.

Mais il n'en est plus de même, si l'on compare les rapports établis comme je vais l'indiquer:

Appelons I S l'indice de saponification du beurre
i s celui de ses acides gras insolubles dans l'eau
I E les acides gras insolubles dans l'eau dosés suivant ma méthode (1er mémoire).
S E les acides solubles dans l'eau dosés aussi suivant ma méthode.

Si l'on établit les rapports suivants:

$$\frac{I\ S}{S\ E} = R^1 \text{ et } \frac{i\ s}{I\ E} = R^2$$

on constate que les beurres purs donnent

R^1 toujours inférieur à R^2

tandis que les beurres cocotés à 5% seulement donnent le contraire: R^1 supérieur à R^2
si l'on ajoute 10% d'um mélange à parties égales de coco et de margarine, R^1 reste encore le plus souvent supérieur à R^2 malgré la margarine ou les deux rapports deviennent au plus égaux.

Mode opérative

Je vais indiquer rapidement comment j'isole une quantité suffisante d'acides gras insolubles dans l'eau pour déterminer en suite leur indice de saponification.

Il consiste à suivre la technique exposée dans le premier mémoire, avec cette différence, que je prélève 10 grammes de beurre fondu et filtré au lieu de 5 grammes; prends un ballon jaugé à 300 au lieu de 150, je saponifie avec 50 cent. cubes de liqueur de saponification au lieu de 25, et j'ajoute 34 cent. cubes d'eau au lieu de 17, pour amener le titre alcoolique à 56°5.

Après la filtration à la température de 15°, je dose les acides gras solubles dans l'alcool à 56°5 et ceux solubles dans l'eau, sur le filtratum, exactement comme je le fais savoir dans le ler mémoire. Ce qui reste de liquide filtré est introduit dans un bécherglass de 250 et évaporé au bain-marie jusqu'à réduction au volume de 45 cent. cubes. La liqueur concentrée, sur laquelle les acides gras insolubles dans l'eau surnagent, est versée étant chaude, (car la filtration est alors plus rapide) sur un petit filtre sans plis que l'on a préalablement échauffé lui-même, en le remplissant d'eau bien chaude.

Après avoir lavé le bécherglass avec de l'eau chaude, pour entraîner le plus possible d'acides gras qui y adhèrent, ceux-ci sont lavés aussi 4 ou 5 fois sur le filtre avec de l'eau bien chaude.

On laisse alors le filtre s'égoutter 2 ou 3 minutes, puis à l'aide d'un tube de très petit diamètre, et à extrémité légèrement

effilée, on prélève les acides gras retenus sur le filtre, pour les introduire dans un petit ballon taré (1).

Une nouvelle pesée fait connaître le poids de ces acides.

On les dissout par addition de 20 cent. cubes d'alcool neutre à 90° 95° et après agitation on prend l'acidité en présence de 2 gouttes de solution de phtaléine, en se servant de potasse déci normale dont on cesse l'addition, dès que se manifeste le virage au rose très faible.

Supposons avoir opéré sur 0 gramme 453 d'acides gras et que leur saturation a nécessité 20 cent. cubes de potasse décime; l'indice de saponification sera donné par le calcul ordinaire à savoir:

$$\frac{20 \times 0.0056}{0.453} = 247 \text{ d'indice de saponification.}$$

Le tableau suivant donne une idée des renseignements que peut fournir l'application des opérations que j'ai citées.

Il est bien certain que si l'on peut ainsi découvrir 5% de coco, on comprend qu'à fortiori, il sera aisé d'en trouver une teneur plus élevée; le rapport R^2 pourrait être utilement cherché; du reste sa détermination ainsi que l'on a pu s'en rendre compte, peut se lier à la pratique de ma méthode rapide d'analyse des beurres (voir 1er mémoire).

	R^1	R^2	
B.P.....	38	43	
B.C.....	40	35	abréviation
B.CM.....	41	38	B.P. —beurre pur
B.P.....	34	37	B.C. —beurre÷5% de coco
B.C.....	36	34	BCM. —beurre÷5% de coco÷5%
BCM	38	36	margarine

(1) Il est bien de chauffer un peu ce tube, pour que les acides ne s'y solidifient point.

THE CHEMIST IN THE SERVICE OF THE PACKING HOUSE

PAUL RUDNICK

Chicago, Ill.

There is perhaps no other industrial line of work embracing so great a variety of subjects as packing house chemistry. This work may vary from the routine analysis of occasional samples of a few fats and fertilizer materials such as blood, bone and tankage, often performed under contract by a commercial chemist, to the great variety of work performed in the laboratories of the larger packing houses by a corps of chemists and trained helpers.

Confining the subject under discussion to the larger laboratories, the work may be classified in a general way into three fairly distinctive lines, namely analytical, research, and consulting work.

The analytical work embraces two principal lines; first, the examination of purchased materials, so far as they can be purchased on specifications involving chemical control, and second, the control of the finished manufactured products, often including the various stages of manufacture as well. Under the head of purchased materials may be mentioned, for example, steel, iron and cement for construction work; paper, tin plate, wood, fiber board, jute, and burlap, for containers or wrapping purposes; and such raw materials as edible and inedible fats and oils, phosphate rock, and potash salts, which enter into the manufacture of finished goods.

Very often unusual specifications have to be made for purchased materials, as for example in the case of special coverings or special containers for meats which have to withstand the deteriorating influence of tropical, humid climates.

In the control of products manufactured in the packing house, one of the principal aims in the case of edible products is, of course, to comply fully with the letter and spirit of the foreign and

domestic laws, including the Meat Inspection Regulations, the Foods and Drugs Act, and the laws of the various individual states. It is also important, however, that the product shall suit the consumer, who often enough seems to know better what he doesn't want, than exactly what he does want. Local customs, likes, dislikes and often prejudices on the part of the consumer and retailer must be met, and as in other selling transactions, this is often possible only by an analysis or examination of a sample of the product which suits the consumer.

In the long list of work on edible packing house products may be included the control of the fresh, cured, smoked, and canned meats, sausages, edible fats, and the processes by which they are produced. To this may also be added the animal products, used chiefly for medicinal purposes, such as pepsin, pancreatin, desiccated glands and their active principles.

Aside from the routine analytical work there are often special analytical problems presented in the control of manufacturing processes. In the manufacture, for instance, of pemmican for polar expeditions it is absolutely necessary to have not only a well-balanced ration of protein, fat and carbohydrates, but the percentage of moisture in the meat, the sweetness of the meat and fat, the amount of salt, spices, sweetening, etc., must be rigidly controlled. Again, in an emergency methods can and must be changed or adapted to meet the situation. Some years ago, for example, it became necessary to determine the absence of boron compounds in not less than 500 samples of cured meat daily. To incinerate and extract such a number of samples daily was impossible with the equipment and help at hand. A number of comparative analyses confirmed the idea that the qualitative test for boric acid could be made on the brine in which the meats had been cured by simple treatment with hydrochloric acid, and direct application of the Goske method, the results being just as satisfactory as if the determinations had been made on the ash of the meat. In this manner it was easily possible for one man to carry on the work at the rate of 500 samples daily.

In the case of soaps, glues, fertilizers, inedible fats and oils, and other inedible products such partial or complete analyses are made as will assure the selling department that the product

will fully meet with the requirements of the purchaser and his specifications, or in the case there are no such requirements or specifications that the products will prove acceptable for the purposes for which they are intended. In a very large part of the work the highest accuracy must be sacrificed to a certain extent to the necessary speed. For instance, it is far more important to avoid per diam penalties or so-called demurrage charges on cars held on the track, to secure cash discounts on materials bought subject to analysis, to avoid costly interruptions in manufacturing processes, the adjustment of which depends on analyses made on the finished product, or to furnish a telegraphic quotation on the day of the inquiry, than to employ unnecessarily refined methods of analysis.

Where so much analytical work must be done, economy of materials and of operation becomes a very important matter. It is necessary, for instance, to arrange the apparatus, for determinations which have to be made in great numbers, in the most compact, simple and convenient form possible, so that the operator will not have to waste time in carrying out the different steps of his work. The expense of reagents can often be materially reduced. For example, the potassium sulphate for nitrogen determinations can be bought from the wholesale druggist in the powdered form in which it is used in medicinal preparations, much more cheaply than the so-called chemically pure article of the chemical supply houses. The only requisite in this case is its freedom from nitrogen, and this must be determined in any event by blank determinations. Again, ammonium nitrate, which is used so largely in phosphoric acid determinations, can be purchased at a relatively low price, if the specifications regarding its purity be limited to absence of phosphates.

It may not be amiss to refer to a mistaken notion which seems to have grown up regarding the value of such analytical work, a notion which has crept even into undergraduate life, namely that analytical work is something to be shunned because of the lack of opportunity for advancement. Nothing could be further from the truth. There is no more valuable training for the industrial chemist and chemical engineer than that which he can obtain in such work as has been described above. If he will

stop to realize what an important bearing even the simplest routine operation may have on manufacturing processes and how often large money values are involved and depend on the faithfulness with which such a simple routine determination is carried out, then his work will take on a new meaning, and actual experience shows that he will make a better man in every way, other things being equal, than the one who lacks such experience and training. Advancement simply depends upon whether or not he is content to remain a routine analyst.

Little can be said regarding the research work which falls to the lot of the packing house chemist. Like all industrial research work it is, from its very nature and purpose, confidential in character, although more frequently than ever before, certain phases of it may be of sufficient scientific interest to bear publication without destroying the value of the general proposition at hand. If this is so, it will doubtless be due to the fact that the methods employed are becoming more and more scientific and less empirical than they have been.

It is out of the question to indicate even in the briefest way the variety of subjects considered in the consulting work required of the packing house chemist, except perhaps, to say that the executive heads, general and department superintendents, engineers, architects, attorneys and many others daily refer questions to the chemist for answer. Many of these subjects are of the greatest interest, sometimes involving careful and extended experimental work, at other times a carefully planned series of analytical determinations or of microscopical or bacteriological work. Occasionally, however, emergencies arise where decisions must be made on the spur of the moment without time for a search of the literature or for actual experiments, otherwise the time for decision would be past.

In conclusion, I cannot emphasize too strongly a fact quite commonly overlooked by the chemist, namely that his work is of no value to the business man unless results, conclusions, judgments, or opinions be reported in simple, clear and concise language, avoiding as far as possible the use of technical, involved, or indefinite expressions, or conditional statements.

AN INVESTIGATION ON THE MANUFACTURE OF TEA

By S. Sawamura
College of Agriculture, Imperial University, Tokyo

I. Effect of Steaming on the Activity of the Enzyms of Tea Leaves

In green tea leaves there are present abundant oxydising enzym, wherefore Mann in India holds an opinion that oxydising enzym is one of the factors which determine the quality of tea. In the manufacture of green tea, however, oxydising enzym of tea leaves is killed by steaming, because when it is active the green color of tea leaves can no more be retained. The author[1] found in another investigation that the formation of some aroma of manufactured tea, which takes place usually during the rolling of tea leaves, is due to the action of a certain enzym on a certain compound of tea leaf. Hence if steaming kills all the enzyms of tea leaves the production of aroma may be more or less hindered.

In 1909 I tried to know whether all the enzyms of tea leaves lose activity by steaming in the usual manner. In these trials green leaves were steamed in the usual manner, respectively for 30 seconds, 50 seconds and one minute, and the steamed leaves as well as the unsteamed were crushed and extracted with 40% alcohol. The extracts are precipitated with ether-alcohol and filtered. The precipitates were washed with alcohol and again dissolved in water. The solution gave no reaction with Fe_2Cl_6, proving the absence of tannin. Oxydising enzyms were tested with guayak tincture, and guayacol and H_2O_2, by which the solution obtained from unsteamed leaves showed the characteristic reaction, while the steamed did not. Steaming for 30 seconds killed oxydising enzyms completely. In another trial tea leaves steamed for 20 seconds were tested for the presence of oxydases, and a faint reaction was observed. From these

[1] Bulletin of Agric. Exp. station, No. 1.

facts we know that the oxydising enzyms of tea leaves lose activity when they are steamed only for 30 seconds.

I tried then to see whether the enzyms other than oxydase lose activity by steaming for a short time. Preliminarily I detected diastase in tea leaf by the following manner. Green tea leaves were crushed in a mortar and extracted with 40% alcohol. To the extract ether-alcohol was added, and the precipitate thereby formed was washed and again dissolved in water. In this solution tannin was removed by hide powder and putrefaction was prevented by the addition of thymol. It was filtered, and the filtrate which gave no reaction with Fe_2Cl_6 and did not reduce Fehling's solution, some boiled starch and thymol were put in. The solution after having been kept at 40° C. for 4 days, reduced Fehling's solution considerably. We confirmed by this trial that diastase of tea leaves can be detected in this manner.

The tea leaves steamed for 30 seconds, in which oxydase was completely killed, reduced also Fehling's solution when treated in the same manner. Hence we know that oxydase is much more sensible than other enzyms such as diastase and it is highly probable that some enzymatic actions take place in the first stage of rolling tea leaves, and the production of some fine aroma is due to them. In practice, therefore, steaming of tea leaves must be so regulated as to kill only oxydising enzyms but not other enzyms.

II. Effect of Rolling on the Solubility of Tea

Whether the object of rolling tea leaves in the manufacture of green tea is to give tea a fine shape or to press out the juice in order to accelerate the desiccation of the leaves, or to break the cells in order to increase solubility is, as far as I know, not yet decided. According to the investigation of Dr. Kozai[1] the solubility of green tea was little increased by the manufacture, but Rombe and Roman's experiment[2] showed on the contrary the decrease of soluble tannin and thein.

To settle this question I made an experiment in 1905, in which

[1] Bulletin of College of Agriculture and Dendrology No. 7.
[2] König. Chemie der Nahrungs und genussmittel B. II.

fresh tea leaves, picked at a sheltered tea garden, were divided into three parts, and one of them was steamed and dried without rolling which served as control; the second part was prepared into green tea (Gyokuro), and the third part into Tencha, which is usually prepared without rolling the leaves. The infusion of these three kinds of tea was found to be as follows:—

	Control	Tencha	Gyokuro
Color	light	deeper	deepest
Flavor	weak	stronger	strongest
Taste	faint	good	best

The reaction of the infusion with Fe_2Cl_6 was not the same in three kinds; that of Gyokuro produced deep black color, while control and Tencha a very faint black color. The solubility of tea was determined as follows:—400 cc. of boiling water were poured on 10 gr. of the powdered sample which had been kept at 100° C. for an hour. It was filtered after leaving it to stand for 5 minutes and washed on filter with 100 cc. of boiling water, and soluble matters were estimated in it.

The composition of the control tea was as follows:—

In 100 pts. of air dry substance	
water	6.215
In 100 pts. of dry substance	
Crude protein	41.984
Albuminoids	28.252
Ethereal extract	9.042
Crude fiber	12.012
Nitrogen free extract	14.101
Thein	3.529
Tannin	15.968
Crude ash	6.883
Total nitrogen	6.717
Albuminous nitrogen	4.520
Thein nitrogen	0.934
Amide nitrogen	1.263

The soluble constituents of the three samples were as follows:—

IN 100 PTS. OF DRY MATTERS

	Control	Tencha	Gyokuro
Dry matter.....	34.057	34.130	33.862
Tannin.....	7.083	6.939	6.477
Thein.....	3.124	2.996	3.088
Ash.....	5.249	5.373	5.197

According to these results Gyokuro, which was prepared by rolling the leaves, showed no greater solubility than the other two. Soluble tannin decreased in Gyokuro probably in consequence of oxydation during the rolling.

In the other experiment I determined solubility of three samples in a different manner. 10 gr. of whole, not powdered sample were put in a beaker, and after keeping it at 100° C. for an hour 200 cc. of boiling water were poured on and filtered through glass wool after leaving it to stand 5 minutes. In the filtrate dry substance, crude protein, tannin, thein and ash were estimated. They were as follows:—

	Control	Tencha	Gyokuro
In 100 part of air dry substance			
Water.....	8.375	7.953	7.638
In 100 pts. of dry matter there were soluble			
Dry matter.....	16.076	21.190	29.233
Nitrogen.....	1.885	2.141	2.313
Tannin.....	0.659	1.312	5.492
Thein.....	1.975	2.243	2.804
Ash.....	3.405	4.411	4.385

	Control	Tencha	Gyokuro
In 100 pts. of each constituent there were soluble			
Dry matter.....	17.545	23.021	31.656
Nitrogen.....	28.068	31.869	34.427
Tannin.....	4.127	8.216	34.374
Thein.....	55.965	63.559	79.453
Ash.....	49.750	64.083	63.708

The increase of solubility compared with the control was found to be as follows:—

	Tencha	Gyokuro
Dry matter.....	5.476	14.111
Nitrogen.....	3.801	6.359
Tannin.....	4.089	30.247
Thein.....	7.594	23.488
Ash.....	14.333	13.958

We see that, when the whole, not powdered samples were used, there were greater increase of solubility in the rolled leaves. Hence we may conclude, that the rolling of tea leaves has the effect of increasing easily soluble matter by crushing the cells and pressing out the juice and making it dry on the surface of the leaves.

Second experiment on the same subject was carried on in 1906 with tea leaves picked in unsheltered tea garden. The leaves were divided into two parts, and one part was dried after steaming and served as control, and the other part prepared into green tea. The infusion of the two samples was found to be as follows:—

	Control	Green Tea
Color.....	For lighter	Common
Flavor.....	Nearly null	Good
Taste.....	Faint	Good

The composition of the original leaves was found to be as follows:—

In 100 pts. of air dry substance	
water	6.008
In 100 parts of dry substance	
Crude protein	33.209
Ethereal extract	25.656
Tannin	18.889
Thein	3.266
Ash	5.719
Soluble matter	44.525
Total nitrogen	5.313
Thein nitrogen	0.864

The solubility which was determined in whole, not powdered samples, was found to be as follows:—

	Control	Green tea
Dry matter	9.879	26.692
Nitrogen	0.969	1.410
Tannin	4.883	12.802
Thein	1.995	2.136
Ash	1.383	3.077

	Control	Green tea	Increase in Green tea
In 100 pts. of each constituent there were soluble			
Dry matter	22.119	59.948	37.829
Nitrogen	18.227	26.531	8.304
Tannin	25.850	67.778	41.928
Thein	61.074	65.403	4.329
Ash	24.186	53.811	29.625

The result of this trial agreed with that of the former one, showing the increase of easy solubility in the rolled leaves. Hence we may conclude, that the chief effect of rolling tea leaves is the increase of easy solubility of the constituents. The desiccation of the leaves will also be accelerated by rolling by pressing out the juice from the interior of the cells. From these facts we are justified in testing tea-infusion to take whole, not powdered sample, and to infuse it only for a few minutes. Total solubility as was determined in the usual method is not of much use for practical purpose.

III. The Effect of Firing on the Chemical Composition of Tea

Green tea as well as black tea are usually refired some days later after the manufacture. By refiring the flavor is much improved, but the infusion becomes usually darker in color. In 1908 and 1909 I made some investigation on the effect of refiring on the quality and composition of tea. I kept respectively green tea and black tea at various temperatures for one hour and then analyzed. Tannin was estimated by Löwenthal's method and thein by Mulder's method. Solubility was determined by infusing 2 gr. of whole tea leaves in 400 cc. of distilled water for 2 hours, and after 100 cc. of water had been added it was filtered. The temperature used for firing, the color and flavor of the infusion and the color of the infused leaves were found to be as follows:—

1908

1. GREEN TEA

No.	Temperature	Color	Flavor	Color of the infused leaves
1	Control (not fired)	Little lighter than No. 3	Weaker than No. 2	Greenish yellow
2	61°C	Nearly same as No. 3	Best	
3	82°C	Best	Little too strong	
4	101°C	Rather red	Bad smell	Little reddish
5	123°C	Reddish than No. 4		Reddish
6	140°C	More reddish No. 5		
7	160°C	Reddish		Blackish brown

2. BLACK TEA

No.	Temperature	Color	Flavor	Color of the infused leaves
1	Control (not fired)	Lighter than No. 2	Weaker than No. 2	
2	62°C	Lighter than No. 5	Weaker than No. 3	Brown
3	81°C	Lighter than No. 1	Best	
4	101°C	Lighter than No. 3		
5	119°C	Most reddish	Bad smell	
6	141°C	Lighter than No. 4		Blackish brown
7	156°C	Lighter than No. 6		

1909

1. GREEN TEA

No.	Temperature	Color of infusion	Aroma of infusion	Taste of infusion	Color of the infused leaves
1	Control (not fired)	Faint	Weak	Weak	
2	60°C	Best	Weak	Astringent	
3	70°C	Lighter than No. 4	Best	Good	Usual
4	80°C	Lighter than No. 2	Good	Best	
5	90°C	Reddish	Bad	Bitter	
6	100°C	Worst		Most bitter	Little burnt

2. BLACK TEA

1	Control (not fired)	Not clear	Faint	Weak	
2	60°C	Light		Good	
3	70°C		Best	Best	Usual
4	80°C	Best	Weaker than No. 3	Weaker than No. 3	
5	90°C	Worse than No. 4	Bad	Bad	
6	100°C	Blackish			Blackish

The chief constituents of the tea were found to be as follows:—

1908

1. GREEN TEA

No.	Temperature	In 100 pts. of air dry subst.	In 100 pts. of dry substance				
		Water	Tannin	Thein	Solubility	Soluble tannin	Soluble thein
1	Control (not fired)	5.228	15.690	3.210	37.458	11.724	2.506
2	61°C	4.633	.....	.....	36.786	11.906	2.600
3	82°C	3.158	.....	.....	35.417	11.785	2.601
4	101°C	1.383	14.602	3.101	35.553	11.688	2.444
5	123°C	2.045	.....	.....	37.364	11.096	2.455
6	140°C	2.453	.....	.....	35.162	10.400	2.287
7	160°C	3.005	13.248	3.098	29.898	6.470	2.317

2. BLACK TEA

1	Control (not fired)	4.445	8.575	3.075	27.393	4.045	2.518
2	62°C	3.985	.....	.....	27.027	4.151	2.676
3	81°C	3.703	.....	.....	27.415	4.390	2.290
4	101°C	2.293	7.247	3.130	27.020	4.450	2.705
5	119°C	4.875	.....	.....	26.281	3.682	2.477
6	141°C	2.230	.....	.....	24.957	2.594	2.443
7	156°C	1.460	5.797	3.135	22.757	1.961	2.500

1909

1. GREEN TEA

No.	Temperature	In 100 pts. of air dry subst.	In 100 pts. of dry substance				
		Water	Tannin	Thein	Solubility	Soluble tannin	Soluble thein
1	Control (not fired)	5.157	15.857	3.077	37.557	11.621	2.191
2	60°C	4.512	15.844	3.134	37.252	11.883	2.425
3	70°C	3.903	.....	.....	37.463	11.936	2.483
4	80°C	3.168	15.418	3.209	36.455	11.711	2.536
5	90°C	2.105	.....	.....	35.569	11.236	2.442
6	100°C	1.844	14.586	3.061	24.666	11.451	2.487

2. BLACK TEA

1	Control (not fired)	6.320	8.484	3.165	27.556	3.917	2.348
2	60°C	4.541	8.632	3.163	27.137	3.844	2.456
3	70°C	3.574	.....	.....	27.378	3.955	2.403
4	80°C	3.218	7.402	3.147	27.098	3.979	2.407
5	90°C	2.271	.....	.....	26.727	3.479	2.221
6	100°C	2.049	7.093	3.046	26.475	3.283	2.200

From these results we may conclude, that green tea is improved in quality by being fired at 70° C. for one hour, and temperature higher than 70° C. spoils both the flavor and color. The optimum temperature for firing black tea lies little higher than for green tea; viz. 80° C , and like green tea higher temperature spoils the flavor and color. By refiring tannin and thein decrease more or less, probably the former being due to oxydation and the latter to volatilization. Solubility increases little when tea is not strongly heated, but when temperature is high total soluble substance and tannin decrease remarkably. Therefore in firing tea temperature must of course be properly applied. If it is too high, the quality of tea is much deteriorated.

WHEAT FLOUR. A MONOGRAPH

HARRY SNYDER, B. S.
Minneapolis, Minn.

In considering the chemical composition of a flour, its moisture content and the basis upon which the results are reported, are matters of first importance. Flour freshly milled may contain from 12.50 to 13.50 per cent of moisture. When stored under the best conditions its moisture content appreciably decreases. Often the chemist receives for analysis a small, over-dried sample sent in paper envelope. This sample may contain less than 8% moisture. Comparison of such a sample with one freshly milled is inconsistent unless the necessary corrections for differences in moisture content be made. This difference in moisture content, unless corrected, disturbs comparative analytical results and is the occasion of much unfavorable comment as to the value of a chemical analysis of flour.

The extent to which a variation of 6% in moisture may affect results is large. A freshly milled flour with 13.50% moisture and .40% ash, would when dried to 7½% moisture show nearly .43% (.427) ash.

The maximum standard of 13.50 per cent of moisture adopted by the U. S. Department of Agriculture is reasonable when the wide range in moisture content of wheat is considered. As an illustration of this range, 278 cars of wheat tested for moisture by myself during the present year showed 48 cars with less than 13.5 %moisture and 230 cars with more than 13.5% moisture.

The question of the moisture content of flour is a disturbing factor not only in chemical analysis but also in the matter of weight of a package. Any loss of moisture causes a proportional loss of weight. Since the Government has established the maximum moisture content of flour at 13½%, that necessarily establishes the minimum dry matter at 86½% and in turn determines the approximate tolerance allowable for shrinkage in

weight. To illustrate: if a 98-lb. package of flour after storage and handling weighs 97 lbs. and is carefully sampled and its moisture content found to be 11½%, it means that the 97 lbs. on an 11½% moisture basis contains 85.84 lbs of dry matter. If the flour had been packed with 13.50% the 98-lb. package would have contained 84.77 lbs. of dry matter. As it is, it contains about one pound of dry matter or dry flour more than is called for on the minimum dry matter basis and hence cannot be considered as short in weight. On the basis of dry matter it is not short in weight and when packed it did not contain the maximum moisture content allowed by the Government standard. Hence in the consideration of both weight and chemical composition of flour the moisture content is a matter of first importance. All comparisons of composition should be made on a uniform moisture basis. The extent to which a flour sample may dry cannot be anticipated and the moisture removed by drying must in turn be added in bread-making. Thus the consumer is in no way defrauded by the drying of flour provided it is packed full weight and without excessive moisture, that is above 13½%. Indeed more water than that would endanger the keeping qualities of the flour and entail loss on the part of the manufacturer. Flour with either an excessively low or high moisture content is not normal flour.

Over-dryness of flour may affect the analytical results by translocation of soluble ingredients. In the case of a sample of flour freshly milled with .40% ash, about one-fourth is mechanically combined—or chemically united with the gluten, one-fourth with the starch and one-half is capable of being dissolved in distilled water. When a flour dries an uneven distribution of the soluble constituents may occur, depending entirely upon governing conditions. Thus if a sample of flour be drawn from only one part of a large flour package it may show an abnormal ash content.

Example of Translocation of Flour Ash.

	Ash Content of Flour	Moisture Content*	
1. Fresh Flour 140-lb. packages	.37+	12.92	.37+
2. Sample from exterior of package after storage 3 mos. in flour store room.....	.41	10.84	.40+
3. Sample from Center of pkg..	.37	11.58	.36+

Drying of a flour accompanied by translocation, and drawing of the sample from a portion only of the package may affect the ash results to the extent of .06 of a per cent. Not infrequently do the results of two laboratories reporting on the same sample of flour show as large and even a larger difference than .06%.

In the determination of the ash of flour other errors that may occur result from: too high a temperature during combustion, incomplete combustion, fusion and occlusion of particles of carbon and failure to make the necessary distinction between crude ash and pure ash. The temperature during combustion should be appreciably below 675°C. and the combustion should be continued until a light grey granular ash is secured, reasonable constant in weight. The ash should not be fused and should be corrected for carbon and combined carbon dioxide. The ash from a refined flour can be obtained quite free from carbon and combined carbon dioxide, so that there is no appreciable difference between the crude ash and the pure ash. But, if the combustion is not made with care, the difference is large.

The main loss caused by high temperatures for combustion is sulphur. This, however, affects the ash percentage less than .01 per cent. There appears to be no loss of phosphorus during the combustion process at low temperature as there is sufficient alkaline matter to form non-volatile pyro phosphates. In fact I have been uuable to obtain any difference in the phosphorus

*Ash on basis of uniform moisture content.

content of a flour from the analysis of the ash, and from the analysis of the residue of the calorimeter where the flour is burned in such a way as to preclude any possible loss of phosphorus compounds.

No official method has yet been adopted by the Association of Official Agricultural Chemists for the determination of the ash of cereals. In the work on flours and cereals by the U. S. Department of Agriculture a just distinction has been made between crude ash and pure ash along the lines laid down by European Chemists.

Methods have been proposed for the complete analysis of the inorganic constituents of plants in which calcium acetate is used to prevent volatilization of sulphur, and then corrections are made for the lime and carbon dioxide introduced by its use. The application to flour of such a method for obtaining ash is not feasible as corrections for carbon dioxide &c. must be made on each individual sample tested. This in turn calls for the combustion of 100 grams of flour so as to get enough crude ash to determine the quantities of impurities to be deducted. Such a method would be impracticable in flour mill work. No common factor could be assumed for correction as a more or less richly carbonated ash of a variable degree of purity is obtained. The acetate introduces one-fourth as much mineral matter as is naturally present in the flour and its use may occasion the introduction of a larger error than* it is intended to correct: viz. volatilization of sulphur.

When made under uniform conditions and by one person, the ash results indicate the mechanical uniformity of a flour to a high degree and are of value in flour mill control work but alone—the ash results are incapable of determining the bread-making value of a flour.

When the ash results of two chemists working on the same sample of flour are compared wide differences may be observed because of variation in moisture content, occlusion, translocation, incomplete combustion and failure to make the necessary distinction between crude and pure ash. These variations are often so large, and unnecessarily so, as to cast much discredit on

* Through formation of carbonates.

chemical tests and their application to determining the value of flour.

Another source of confusion in the interpretation of a flour analysis is the occasional use of the factor 5.7 for converting total nitrogen into protein, instead of 6.25 and then no mention being made of the factor used. In technical scientific investigation the use of special factors for protein determination is necessary and unquestionable, but to use a special factor for wheat and a general factor (6.25) for all other foods is inconsistent. From a nutritive point of view the 6.25 factor for wheat is more correct than 5.70 because the wheat proteins are concentrated in nitrogen containing 17.50% against the general average of 16.25. It is the nitrogenous part of the molecule which gives the unique food value to the proteins, and in the wheat proteins the consumer gets more of this material. In fact the 5.7 factor assigns too low a nutritive value to wheat. Wheat proteids are too concentrated in distinctive nitrogenous material to be assigned so low a percentage value—when compared with other foods where the proteins are of lower nitrogen content. It is the quality of the protein that determines its nutritive value as well as the amount, and in wheat the proteins are of strong nitrogenous character.

The general method for the determination of crude fiber is not satisfactory for determining the fiber content of flour, as the action of the acid and alkali solutions for the removal of non-fiber materials is not complete. By extracting the flour with 70% alcohol, after extraction with ether, better results are obtained as the gliadin is removed, foaming is prevented and the material is in better mechanical condition for extraction with acid and alkali solutions. This extraction with alcohol is beneficial in determining the fiber of wheat products.

In conclusion, it may be said that the chemist can do most in the way of flour investigations by making a study of bread-making processes and the factors which control them. Flour making is distinctly a mechanical process and the whole tendency of modern flour manufacture is in the direction of producing cleaner flour.

Since wheat flour takes such an important part in the dietary and because it supplies such a large amount of nutrients at com-

paratively low cost, it is consistent that the efforts of the chemist be directed toward encouraging the farmer to raise the best of bread wheats in the most approved ways through scientific agriculture, and that a broader knowledge be secured and generally disseminated concerning the principles of bread-making and the nutritive value of bread and foods in general. In this work the food chemist must necessarily take the leading part. If indifferent, he is not doing his duty.

ON SOME DRIED MILKS AND PATENT FOODS

By A. W. Stewart, D.Sc.
West Hampstead, London, N.W., England

Whether dried milks, infants' foods and other milk food preparations are becoming more popular or that mothers find it more convenient to bring up their children on these products, the fact remains that there is a steady increase in the number of these preparations. Although some are of a wholesome nature there are on the market nevertheless a large number whose chief ingredient is starch, contained in such a proportion as to be totally unsuitable for infants. Artificial products cannot effectually replace what nature has supplied. The characters of human milk are such that its imitation seems almost irrealisable. It is true that by diluting cows' milk with water and adding lactose and cream, an article can be produced much resembling human milk, but it has not those properties that render mothers' milk the ideal food for children. Sommerville pointed out the value of dried milk for infant feeding on account of its relative sterility and the absence of a dense clot in the infant's stomach. When one studies the results of the analyses of the infants' foods on the market, the unsuitability of the majority of them is greatly in evidence. The Australian Food Standards Committee 1906, recommended that "Infants' food shall contain no woody fibre, no preservative substance and no mineral substance insoluble in water; and unless described or sold specifically as a food suitable only for infants over the age of seven months shall, when prepared as directed by an accompanying label, contain no starch and shall contain the essential ingredients of and conform approximately in composition to normal mothers' milk." Apart from the high starch percentage of a number of brands, there are not many which form even in 10% solutions anything approaching a homogeneous solution. In several cases this was not possible, the result being a thick

pasty mass caused by the swelling and cohesion of the starch. The small % of fat is an outstanding feature of inferior brands; they are deficient in the chief body fuel namely fat. Abundance of fat should be the main characteristic of the diet of infancy just as abundance of carbohydrates is the chief feature of the adult and laborious life. The frequent connection between rickets and deficiency in fat is an undeniable clinical fact. Again, the desiccating process destroys the enzymes always present in raw milk and to which its anti-scorbutic properties are generally ascribed.

Preparation

Until lately the process of separating the solid and liquid constituents of milk was too costly to render the manufacture of "dried milk" a profitable industry. The machine used in the "Just-Hatmaker" process appears to give the most satisfactory results; it consists of two large metallic drums 28 inches in diameter and 5 feet long, mounted horizontally in a framework with a space of about $\frac{1}{8}$th inch between them. High-pressure steam, admitted to the drums through axial pipes, raises their surfaces to a temperature of 220° F. The milk is allowed to flow in thin streams over the revolving drums, the heat of which quickly evaporates the water. A coating of solid matter gradually forms which is scraped off by a knife and falls into a receptacle. The milk is not boiled, though completely sterilised by the heat. A slight decomposition of the proteins and fat probably takes place.

Classification

1. Dried milks and milk products; they consist of milk evaporated and dried, with or without the addition of lactose and fat. They are free from starch. The results are given in table I.

2. Farinaceous and malted foods; in this are included foods containing either malt or starch or both. In many cases the diastase was not active. The results are given in table II.

3. Miscellaneous products, oats, barley, etc., for growing children. The results will be found in table III.

Methods of Analysis

Water: This was estimated by drying 10 grams of the sample at 100° C. for 4–6 hours.

Ash: The residue was incinerated and weighed in the usual manner.

Total P_2O_2: Where it was desirable to estimate the phosphates, a titration with N–10 uranium nitrate using $K_4Fe(CN)_6$ as indicator was made.

Total proteins: This was estimated by the usual Kjeldahl process. The factor N x 6.38 was used unless otherwise stated. A verification of this factor was effected by D. Richmond (Analyst, 1908, p. 179). When the nitrogen is present as casein or albumen the factor 6.38 should be used but when its origin is uncertain 6.25 is recommended as a general factor.

Soluble albumen: 10 cc. of a 10% solution are digested with 20 cc. of saturated $MgSO_4$ solution and crystals of magnesium sulphate are added until they no longer dissolve. An excess does not matter, provided the $MgSO_4$ is free from Na_2SO_4. The solution is put aside till the next morning, when it is filtered and washed with a little saturated $MgSO_4$ solution. The filtrate is then treated with Almen's reagent (4 gram tannic acid, 190 cc. 50% alcohol, 8 cc. 25% acetic acid) and the precipitate allowed to settle till the next day. It is then filtered, paper and contents transferred to a Kjeldahl flask and the estimation of the proteins carried out in the usual way.

Lactose and Carbohydrates. These were obtained by difference.

Fat: We utilised 3 methods: (1) Leffmann and Beam, centrifugalisation of fat (2) Adams and Soxhlet process, the latter in certain cases where the Adams process could not be applied owing to the product not forming a solution with water (3) Werner-Schmidt. The last named is the only reliable method combining accuracy with rapidity. The Adams and Soxhlet extraction were often found to be unreliable. This is in agreement with McLellan (*Analyst*, 1908, p. 353); he found that the incompleteness of the extraction of the fat is due to the coating of the fat globules in the milk during the process of evaporation with an impermeable substance which prevents the solvent from penetrating. He found that it was possible to completely

extract the fat from dry milk in a soxhlet apparatus if the sample was soaked overnight, then extracted for 8 hours and allowed to soak again overnight and finally extracted for 1 to 2 hours. That the results obtained by the Adams process are too low is further borne out by Siegfeld (*Molkerei Zeitung*, 1909, N. 25), Grünhut (*Zeit f. Anal. Chem.*, 1911, p. 649), and Anton Burr (*Milchwirtschaftl. Zentralbl.*, 7, p. 118). The latter found the Werner-Schmidt and Rose-Gottlieb processes to give good results, the last named requiring special conditions to be complied with according to D. Richmond (*Analyst*, 1908, p. 389).

Physiological Fuel Value: The results are expressed in large calories per 100 grams of the sample. The factors used were fat 9, protein 4, and carbohydrate 4, these being the physiological fuel values of food constituents.

Nutritive Ratio: is the ratio of proteid to carbohydrate and fat, i.e., the ratio of nitrogenous to non-nitrogenous nutrients compared on the basis of fuel values. It is deduced from the formula (Sherman, Chemistry of food and nutrition 1911):—

$$\frac{\text{carbohydrates} + 2\frac{1}{4}\,\text{fat}}{\text{proteins}}$$

No preservatives (formaldehyde, boric acid) were found in any of the samples analysed.

TABLE I

DRIED MILKS AND MILK PRODUCTS EXEMPT FROM STARCH

Mark		Water	Fat	Total Ash	Total Proteins	Soluble Albumen	Lactose	Fuel Value	Nutritive Ratio
Dried human milk	(1)	.....	30.02	2.46	18.18	10.00	49.32	540	1:11
Cow and Gates	(2)	3.36	25.00	6.32	27.5	2.23	37.82	486	1:6
id.	(3)	4.58	10.00	6.98	30.90	2.23	41.54	433	1:4
id.	(4)	4.76	1.86	8.43	33.75	2.74	51.20	356	1:1.8
Trumilk	(5)	1.46	30.00	5.57	27.69	4.91	35.28	521	1:7
id.	(6)	7.3	1.04	7.41	32.85	3.57	51.40	346	1:1.7
Allenbury No. 1	(7)	2.12	18.46	3.80	11.23	1.87	64.39	468	1:14
Allenbury No. 2	(8)	3.70	16.28	3.56	11.55	2.23	64.91	452	1:13
Proteid Huxley A	(9)	17.21	.13	8.30	45.36	.....	29.00	298	1:1
Proteid Huxley B	(10)	14.99	5.62	8.14	50.72	.....	20.53	335	1:1
Neaves Milk Food	(11)	3.64	25.37	4.28	19.65	3.03	47.06	495	1:9
Sanatogen	(12)	8.28	.12	5.46	84.15	.....	.....	337	....
Albulactin		8.70	.....	5.64	85.30 (13)	.....	.....	341	....
Hogg's Protein Nerve Food		11.08	.78	6.30	80.38 (14)	.....	.....	328	....
Robb's Soluble Milk Food 1	(15)	2.52	14.50	3.46	14.67	1.70	64.85	448	1:9
Robb's Soluble Milk Food 2	(16)	4.38	9.83	4.74	18.37	1.70	62.68	404	1:6
Plasmon	(17)	9.71	.52	7.68	75.92	.....	.....	308	....
Majax	(18)	.....	.....	1.03	.....	.....	98.2	392	....
Humanoid	(19)	66.60	11.58	.70	4.78	1.78	16.34	188	1:16

(1) Figures calculated from König: Chemie der Mensch. Nahrungs ü Genussm. (2) Full cream. (3) Half skimmed. (4) Machine skimmed. (5) Full cream. (6) Skimmed. (7) Dried milk with added lactose. (8) Idem. (9) Contains sodium glycerophosphate. (10) Made from milk and eggs with addition of glycerophosphate. (11) Dried milk with added lactose. (12) Milk caseine with glycerophosphate P_2O_5=.71%. (13) Lactalbumin. (14) Milk caseine. (15) Dried milk with added lactose. (16) Idem. (17) Milk caseine P_2O_5=2.94%. (18) Lactose. (19) Concentrated and modified milk.

TABLE I—*Continued*

DRIED MILKS AND MILK PRODUCTS EXEMPT FROM STARCH

Mark		Water	Fat	Total Ash	Total Proteins	Soluble Albumen	Lactose	Fuel Value	Nutritive Ratio
Paget's Modified Milk 1.....	(20)	71.58	7.83	.57	3.12	1.27	16.90	150	1:19
Welford Humanized Milk.....	(21)	88.98	3.36	.24	1.22	.14	6.20	60	1:18
Vitafer.....	(22)	9.85	.48	9.25	71.90	.....	.....	292	....
Cibrola.....	(23)	9.32	.62	10.29	72.35	.....	.....	295	....
Glaxo.....	(24)	1.89	27.0	5.55	24.62	6.06	40.94	505	1:7
Trufood.....	(25)	4.76	13.45	6.88	20.0	3.82	54.91	421	1:6
Norwegian.....	(26)	7.30	.34	7.38	32.15	1.25	52.83	343	1:2
Dutch.....	(27)	4.43	24.0	6.33	24.11	1.60	41.23	477	1:6
id.	(28)	7.75	.86	7.26	35.72	2.86	48.41	344	1:1.5
id.	(29)	5.45	.35	8.17	31.96	8.03	54.07	346	1:1.7
English.....	(30)	5.52	26.87	5.59	23.92	3.21	38.10	490	1:7
id.	(31)	8.0	.80	8.13	34.64	5.18	48.43	339	1:6
American.....	(32)	6.86	1.0	7.88	34.64	4.37	49.62	346	1:1.6
French.....	(33)	4.36	23.55	5.72	25.62	3.75	40.75	477	1:6
id.	(34)	7.36	.73	7.55	34.64	1.35	49.72	344	1:1.6
New Zealand "Defiance"	(35)	3.70	29.70	5.33	26.79	2.76	34.48	512	1:7

(20) idem. An enclosed packet contained .2112 grm. $NaHCO_3$. (21) Supposed to resemble human milk. (22) P_2O_5= 3.97%; milk caseine with glycerophosphate. (23) P_2O_5=3.19%; idem. (25) Modified dried milk. (26) Skimmed. (27) Full cream. (28) Best quality separated. (29) Ordinary qual. separated. (30) Full cream. (31) Separated. (32) idem (33) Full cream. (34) Extra fine qual. separated. (35) Full cream.

TABLE II
MALTED AND FARINACEOUS FOODS

Mark	Water	Fat	Total Ash	Total Proteins	Soluble Albumen	Carbohydrate	Fuel Value	Nutritive Ratio
Horlick's malted milk	3.58	7.65	3.67	14.55	3.04	70.54 (1)	409	1:8
Allenbury malted food No. 3	4.68	.94	1.01	11.80	1.16	81.57 (2)	381	1:7
Milo	3.26	6.40	1.26	12.86	1.61	76.22 (3)	413	1:8
Virogen (4)	7.21	.54	6.15	47.65	.....	38.45	349	1:1
Neaves Food for infants	5.40	.91	.68	13.71	.....	79.30 (5)	380	1:6
Benger's Food	7.47	.82	1.06	12.50	.....	78.15 (6)	370	1:6
John Bull Malt-and-Milk 1	1.42	17.0	4.43	19.14	.....	58.01 (7)	461	1:7
John Bull Maltassa 2	4.20	1.00	1.58	13.93	1.78	79.29 (8)	381	1:6
Miriam's Food	10.41	4.25	1.03	11.87	.....	72.44 (9)	375	1:8
Ridge Patent Food	4.70	1.34	.98	11.25	.....	81.73 (10)	384	1:8
Neaves Health Diet	5.41	15.13	3.10	19.07	.....	57.29 (11)	441	1:1
Chapman malted food	9.93	1.86	1.36	12.25	.....	74.60 (12)	364	1:1
Mellin's Food	2.81	.21	3.18	14.00	.....	79.8 (13)	377	1:6
Mellin's Lactoglycose	4.94	7.10	4.06	18.37	3.21	65.53 (14)	400	1:6
Ovaltine (15)	3.98	5.40	3.63	15.31	2.76	71.68	396	1:6
Savory-and-Moore	5.07	1.43	.77	12.93	.....	79.80 (16)	383	1:7
Carnrick's Soluble Food (17)	1.71	1.76	3.58	19.14	5.1	73.81	387	1:4
Chapman's Food	9.37	1.98	1.64	12.75	.....	74.26 (18)	365	1:6
Frame Food	3.37	.46	1.52	13.31	.....	81.34 (19)	383	1:6

(1) Dextrine, maltose, lactose; contains active diastase. (2) Lactose maltose; wheat flour and malt. (3) Dextrine, maltose, lactose, sucrose; Nestle's desiccated milk with baked wheat starch. (4) Caseine with sodium glycerophosphate. Trace of starch. (5) Wheat flour. (6) Wheat flour with pancreatic extract. (7) Maltose, dextrose, dextrine, lactose; active diastase. (8) Starch, maltose, dextrose, dextrine, lactose. (9) Baked wheat flour N x 6.25. (10) idem. (11) Dried milk and wheat flour. (12) N x 6.25. Flour and malt. (13) No starch; a completely malted product. (14) Mellin's Food with dried milk. (15) No starch; malt extract, dried milk, eggs, a little cocoa. (16) Wheat flour N x 6.25. (17) Dried milk with wheat flour and a little malt. (18) N x 6.25. Wheat flour without malt. (19) N x 6.25. Starch, maltose, dextrine, sucrose. Baked wheat flour.

TABLE III

MISCELLANEOUS PRODUCTS

Mark		Water	Fat	Total Ash	Total (1) Proteins	Carbo-hydrate	Fuel value	Nutri-tive ratio
Maltcon	(2)	25.06	1.28	9.54	14.12	50.00	268	1:4
Quaker Oats	.....	9.95	7.43	1.70	15.56	65.36	370	1:7
Plasmon Oats	(3)	8.98	9.28	2.14	18.87	60.73	402	1:6
Plasmon arrowroot	(4)	13.19	.46	2.15	18.06	66.14	341	1:4
Glidine	(5)	7.56	.45	.53	87.27	4.19	369	....
Virol	(6)	24.10	15.00	1.04	3.37	55.49	374	1:40
Cerola	(7)	10.56	1.00	.52	12.50	75.42	360	1:6
Farola	(8)	10.79	1.39	.44	13.00	74.38	362	1:6
Robb's Nursery Biscuits	.....	5.91	5.36	1.01	10.37	77.35	399	1:10
Manhu prepared barley	.....	10.72	1.96	1.24	9.37	76.71	362	1:9
" flaked "	.....	10.66	1.72	1.73	10.18	75.71	359	1:8
" " rye	.....	10.48	1.49	1.64	10.75	75.64	359	1:7
" " wheat	.....	10.16	1.86	1.64	11.43	74.91	362	1:7

(1) N x 6.25. (2) Starch maltose, dextrose; extract of yeast. (3) Oats with Plasmon. (4) Arrowroot with Plasmon. (5) Pure gluten N x 5.7. (6) No starch; bone marrow, yolk of eggs, malt extract, flavored with lemon juice. (7) Granulated wheat. (8) Idem in fine powder.

The question of starch in infants' foods

A short perusal of the results tabulated will show that nearly 50% of the samples analysed contained starch. Nearly all of these milk food preparations are intended for infants' food, so that this question is of capital importance in the bringing up of children from birth. This subject was dealt with in an interesting paper by Cautley.—(Lancet, Nov. 6, 1909), amongst whose conclusions are:—

(I) A diastasic ferment is secreted by the salivary glands and pancreas of new-born infants; the salivary secretion however is scanty in young infants and rarely appreciable before the age of two months.

(II) Barley water contains about 2% of starch; mixtures containing this percentgae of starch are not injurious but may be beneficial, for the growth of lactic acid bacilli and the formation of lactic acid are thereby encouraged. These organisms are of undoubted advantage in the prevention of the growth of proteolytic bacteria.

(III) The evil effects of starch in early life are due to (a) excess, (b) its admistration in the form of a more or less insoluble emulsion instead of as soluble starch (c) the substitution of starch for the necessary protein, fat and salts.

Under the title "Patent Foods" an interesting pamphlet has been written by R. Hutchison. The defects of artificial foods may be summed up as follows:—

(I) They are often recommended when there is marked loss of appetite; they do not promote it.

(II) It is often contended for these products that they are more easily digested than natural foods and many of them exist because they are pre-digested. The necessity for peptonizing foods is greatly exaggerated. In pathological chemistry pepsin is almost never absent from the gastric juice unless HCL is also absent. If HCL can be found in the stomach pepsin is sure to be there too; there is therefore little necessity for predigested foods.

(III) The claim often put forward for artificial foods that they enable you to enrich the diet in certain constituents is largely fallacious.

(IV) None of them is worth the money asked for it; some of them contain a ricidulously small amount of nourishment at the price. It is vastly more expensive to rear a child upon one of them than upon fresh or even condensed milk.

ÜBER DIE CHEMISCHE ZUSAMMENSETZUNG DES "SALZBREIES" VON BONITO ("SHIOKARA")

Von U. Suzuki, C. Yoneyama und S. Ōdake,

Landwirtschaftliche Facultät, Kaiserliche Universität, Tokyo, Japan

Zur Bereitung des "Salzbreies" wird der Magen und Darm des Bonitos vom inneren Inhalt befreit, gut gewaschen, fein zerhackt und mit viel Kochsalz vermischt, so dass es einen dicken Brei gibt. Die Leber wird auch manchmal dazu gemengt. Man lässt nun den so bereiteten Brei wochenlang bei Zimmertemperatur stehen und rührt öfters um. Es tritt dabei allmählich die Reifung ein; es entwickelt sich ein eigentümlicher Geruch und Geschmack, und von vielen Leuten, besonders von Sakekennern wird der Artikel als Delikatesse mit Vorliebe genossen. Die an der Reifung des Breies teilnehmenden Mikroben sind bis jetzt nicht untersucht, und die chemischen Vorgänge, die während des Reifeprozesses vor sich gehen, sind auch noch nicht näher erforscht. Nur vermutet man, dass sie den bei der "Shōyu" Bereitung auftretenden ziemlich ähnlich sind. Durch Einwirkung von Mikroben und Enzymen werden verschiedene Stoffe, besonders Eiweissstoffe, allmählich gelöst und abgebaut, unter Bildung von Peptonen und Aminosäuren, die zum Teil weiter desamidiert, oxidiert oder reduziert werden. Es entstehen dabei verschiedene Säuren, Alkohole, Amine u.s.w. Die Zusammensetzung des Breies ist deshalb sehr kompliziert. Es kommen bei verschiedenen Reifestadien verschiedene Stoffe zum Vorschein.

Wir beschränken uns vorläufig mit der Untersuchung der stickstoffhaltigen Bestandteile des käuflichen, gereiften Breies. Das von uns untersuchte Material war aus Odawara bezogen. Es war grau-rötlich-braun gefärbt und reagierte ziemlich stark sauer. Die quantitative Bestimmung gab folgendes Resultat:

In 100 Teilen frischen Breies

Wasser	65.13
Trockensubstanz	34.87

In 100 Teilen Trockensubstanz

Organische Stoffe	30.06
Asche	69.94
Chlor	29.80
(Als NaCl berechnet)	49.18

	In 100g frischen Breies	Gesamt-N als 100
Gesamt–N	1.735	100.0
Eiweiss–N	0.472	27.2
Org.-Basen–N	0.447	25.7
Ammoniak–N	0.131	7.6
Anderes N	0.685	39.5

Zur Isolierung der stickstoffhaltigen Stoffe wurden 4 Kilo Brei aus gepresst. Der Rückstand wurde drei mal mit warmem Wasser (40–50°) extrahiert. Die vereinigten Auszüge, die schwach sauer reagierten, betrugen rund 9 liter. Sie wurden mit 20% iger Tannin lösung gefällt. Der Tannin-Niederschlag (B) wurde abgesaugt und mit Wasser gewaschen. Das Filtrat vom Tannin-Niederschlag wurde mit verdünnter Natronlauge verstezt, bis es schwach alkalisch reagierte. Es entstand dabei eine flockige Fällung (C) in reichlicher Menge. Man saugte davon ab, und setzte dem Filtrat viel Baryt zu, um das Tannin zu entfernen, saugte wieder ab und nach dem Entfernen des Baryts mittelst Schwefelsäure dampfte man bei niederem Druck stark ein. Es schieden sich dabei Tyrosin, Leucin und anorganische Salze aus. Aus heissem Wasser umkrystallisiert, erhielt man zuerst 3 g Tyrosin und von der Mutterlauge desselben 2.1 g Leucin. Beide Aminosäuren wurden nochmals für sich umkrystallisiert und analysiert.

Tyrosin:

0.1604 g Subst. gaben 10.7 c.c. N (16° 760 mm)

		N
$C_9H_{11}NO_3$	Ber.	7.70
	Gef.	7.78

Leucin:

0.1719g Subst. gaben 15.7 c.c. N (14° 758 m.m.).

		N
$C_6H_{13}NO_2$	Ber.	10.07
	Gef	10.71

Die Mutterlauge von Leucin und Tyrosin wurde mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt.

A Der Phosphowolframsäure-Niederschlag

Die aus diesem Niederschlag dargestellte alkalische Flüssigkeit, die freie Basen enthielt, lieferte nach starkem Einengen im Vakuum keine Krystalle, so wurde sie mit Kohlensäure gesättigt und mit Quecksilberchlorid gefällt.

a) Der Quecksilberchlorid-Niederschlag wurde mit Schwefelwasserstoff zerlegt, im Vakuum ein gedampft und mit Pikrinsäure erwärmt. Nach dem Erkalten schieden sich 8.5 g fast reines Lysinpikrat aus, welches aus heissem Wasser umkrystallisiert und analysiert wurde.

0.1414 g Subst. gaben 22.0 c.c. N (13° 766 mm)
0.1491 g " " 0.2094 g CO_2 0.0612 g H_2O
0.4149 g " " 0.2533 g Pikrinsäure

		C	H	N	Pikrinsaüre
$C_6H_{14}N_2O_2$, $C_6H_3N_3O_7$	Ber.....	38.40	4.53	18.67	61.07
	Gef.....	38.34	4.56	18.53	61.05

Im Kapillarrohr erhitzt, zersetzte sich das Pikrat gegen 247° (unkorr.).

Das Platinchlorid-doppelsalz des Lysins waren hygroskapische goldgelbe lange Prismen. Es schmolz bei 205° (unkorr.).

Für die Analyse wurde es im Vakuum bei 100° getrocknet.

0.3014 g Subst. gaben 0.1055 g Pt.

		Pt.
$C_6H_{14}N_2O_2$, H_2PtCl_6	Ber.....	3.500
	Gef.....	35.00

b) Das Filtrat vom Quecksilberchlorid-Niederschlag wurde nach dem Entfernen des Quecksilbers durch Schwefelwasserstoff und der Salzsäure durch Silbernitrat, mit einem Überschuss von Silbernitrat und Baryt versetzt. Der braune Niederschlag lieferte 1.3 g Lysinpikrat.

Die Analyse des gereinigten Salzes gab folgendes Resultat:

0.1313 g Subst. gaben 21.3 c.c. N (20° 760mm)

		N
$C_6H_{14}N_2O_2$, $C_6H_3N_3O_7$	Ber.....	18.67
	Gef.....	18.56

c) Das Filtrat vom Silbernitrat und Baryt-Niederschlag wurde in bekannter Weise mit Phosphowolframsäure gefällt. Aus diesem Niederschlag erhielt man wieder 6 g Lysin pikrat.

0.1374 g Subst. gaben 22.3 c.c. N (21° 763 mm)
0.1526 g " " 0.2160 g CO_2 0.0672 g H_2O

		C	H	N
$C_6H_{14}N_2O_7$, $C_6H_2N_3O_7$	Ber.	38.40	4.53	18.67
	Gef.	38.60	4.89	18.55

B. Der Tannin-Niederschlag (Trytophan)

Der vom wässerigen Extrakt des Salzbreies durch Zusatz von Tannin erhaltene Niederschlag wurde mit 3% iger Schwefelsäure wiederholt verrieben. Ein Teil ging dabei in Lösung. Man filtrierte nun vom unlöslichen Rückstand ab und setzte dem Filtrate viel Baryt zu, um damit Tannin und Schwefelsäure wegzuschaffen. Das vom dabei entstandenen Niederschlag abgesaugte Filtrat wurde mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt.

a) Durch Zerlegung des phosphowolframsauren Niederschlags wurde eine Flüssigkeit erhalten, welche schöne Tryptophanreaktion gab. Wird diese Flüssigkeit mit Essigsäure angesäuert und mit einigen Tropfen Bromwasser versetzt, so entsteht eine rot violette Färbung. Beim Schütteln mit Amylalkohol geht der Farbstoff in das letztere Reagenz über. Um das Tryptophan zu isolieren, wurde die Flüssigkeit mit so-viel Schwefelsäure versetzt, bis sie 5% der Säure enthielt, und mit Hopkinischem Reagenz gefällt. Es enstand dabei eine weisse flockige Fällung, die absesaugt, mit 5% iger Schwefelsäure gewaschen und mit Schwefelwasserstoff zerlegt wurde. Beim Eindampfen des Filtrats im Vakuum schied sich ein Teil des Tryptophans krystallinisch aus. Die Hauptmasse blieb jedoch amorph, so dass das gereinigte Tryptophan nicht zur Analyse ausreichte.

Aus dem Filtrate vom Quecksilbersulfat-Niederschlag des Tryptophans wurde eine Base als pikrinsaures Salz isoliert. Dies genügte auch zur weiteren Untersuchung nicht.

b) Das Filtrat vom Phosphowolframsäure-Niederschlag lieferte, nach der Estermethode verarbeitet, eine kleine Menge Leucin.

C. Der Tannin=und Natronlauge Niederschlag

Wie oben erwähnt, lieferte das Filtrat vom Tannin Niederschlag (B) durch Zusatz von verdünnter Natronlauge, wieder eine reichliche Fällung, die eine nicht unbeträchtliche Menge Basen enthielt. Um die Basen zu isolieren wurde der Niederschlag mit 5% iger Schwefelsäure verrieben, wobei ein grosser Teil in Lösung ging. Die braune Flüssigkeit wurde nun mit einem Überschuss von Baryt versetzt, vom dabei entstandenen Niederschlag abgesaugt, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Nach Zerlegung des phosphowolframsauren Niederschlags in bekannter Weise erhielt man eine alkalische Flüssigkeit, die freie Basen enthielt. Diese Flüssigkeit wurde nun mit Kohlensäure gesättigt und mit Quecksilberchlorid gefällt.

a) Der Quecksilberchlorid-Niederschlag (Histidin)

Aus diesem Niederschlag erhielt man eine alkalische Flüssigkeit, die sowohl starke Paulysche Reaktion, wie auch Biuretreaktion beim Erwärmen gab. Bei Zusatz von Pikrinsäure wurde 0.5 g Histidinpikrat gewonnen, welches aus heissem Wasser umkrystallisiert, im Vakuum bei 100° getrocknet und analysiert wurde.

0.1430 g Subst. gaben 27.8 c.c N (24° 759 mm)
0.1517 g " " 0.2034 g CO_2 0.0457 g H_2O

		C	H	N
$C_6H_9N_3O_2$, $C_6H_3N_3O_7$	Ber.	37.50	3.13	21.91
	Gef.	37.56	3.35	21.77

Die Analyse stimmt also mit dem Histidin pikrat überein. Der Schmelzpunkt war jedoch viel höher als beim gewöhnlichen Histidin pikrat, welches aus den Spaltungsprodukten des Eiweisses dargestellt wird. Im Kapillarrohr erhitzt, wurde es gegen 200° braun und zersetzte sich gegen 210° (unkorr.) unter Schäumen. Es handelt sich wahrscheinlich um eine Isomerie des Histidins. Wegen Mangel an Material konnten wir das optische Verhalten nicht untersuchen.

b) Das Filtrat vom Quecksilberchlorid-Niederschlag des

Histidins wurde mit Silbernitrat und Baryt gefällt. Aus diesem Niederschlag isolierte man eine Base als pikrinsaures Salz, welches 1.5 g betrug. Das Pikrat bestand aus rot braunen blättrigen Krystallen mit dem Schmelzpunkt 225° (unkorr.). Die Analyse gab folgendes Resultat:

0.1171 g Subst. gaben 24.2 c.c. N (20° 760 mm)
0.1372 g " " 0.1715 g CO_2 0.0496 g H_2O
0.2504 g " " 0.1919 g Pikrinsäure.

	C	H	N	Pikrinsaüre
$C_6H_{14}N_4O_2$ $(C_6H_3N_3O_7)_2$ Ber.....	34.18	3.16	22.15	72.47
Gef.....	34.09	4.02	23.64	76.63

Die Analyse stimmt also mit dem Arginindipikrat, nur ist der Gehalt an Stickstoff und Pikrinsäure etwas höher.

c) Das Filtrat vom Silbernitrat und Baryt-Niederschlag (b) wurde wieder mit Phosphowolframsäure gefällt. Der Niederschlag lieferte 2.34 g Lysinpikrat mit dem Zersetzungspunkt 245° (unkorr.).

D. Das Filtrat vom phosphowolframsauren Niederschlag

Das Filtrat vom phosphowolframsauren Niederschlag wurde nach der Estermethode verarbeitet, indem die Phosphowolframsäure und Schwefelsäure durch Baryt entfernt und der Überschuss vom Baryt mittelst Schwefelsäure beseitigt und im Vakuum stark eingedampft wurde. Der zurückgebliebene Syrup wurde nun mit absolutem Alkohol versetzt, mit trockenem Salzsäuregas gesättigt und in bekannter Weise in die freien Estern der Aminosäuren verwandelt. Nach fraktionierter Destillation der Estern, wurden die folgenden drei Fraktionen erhalten:

	Temperatur	Estermenge	Aminosaüren nach der Verseifung
1	bis 75° (20mm)	9.0 g	3.0 g
2	75–100° "	10.5	7.8
3	über 100° "	7.0	6.0

Fraktion 1 bestand aus Alanin. Aus heissem Wasser umkrystallisiert, bildete es farblose Nadeln mit süssen Geschmack und zersetzte sich gegen 270°. Für die Analyse wurde es im Vakuum bei 100° getrocknet.

0.1553 g Subst. gaben 21.3 c.c. N (17° 760 mm)
0.1530 g " " 0.2248 g CO_2 0.1040 g H_2O

		C	H	N
$C_3H_7NO_2$	Ber.	40.45	7.87	15.88
	Gef.	40.07	7.55	16.03

Fraktion II. bestand auch hauptsächlich aus Alanin, nebst einer Kleinen Menge Prolin.

Analyse des Alanins:

0.1476 g Subst. gaben 20.4 c.c. N (21° 751 mm)

		N
$C_3H_7NO_2$	Ber.	15.88
	Gef.	15.62

Kupfersalz des Alanins:

0.2114 g Subst. gaben 0.0703 g CuO.

		Cu
$(C_3H_6NO_2)_2Cu$	Ber.	26.22
	Gef.	26.57

Fraktion III. bestand zum grössten Teil aus Leucin, nebst Alanin und Prolin. Zweimal aus heissem Wasser umkrystallisiert wurde das Leucin in ziemlich reinem Zustande erhalten. Es schmeckte schwach bitter und zersetzte sich gegen 280°.

Analyse des Leucins:

0.1467g Subst. gaben 13.4 c.c. N (19° 762 mm)

	N
$C_6H_{13}NO_2$ Ber.	10.07
Gef.	10.56

Das Prolin wurde isoliert, indem die nach der Verseifung des Esters erhaltenen Aminosäuren mit heissem absolutem Alkohol extrahiert wurden. Der vereinigte alkoholische Extrakt wurde eingedampft und der Rückstand nochmals mit absolutem Alkohol extrahiert. Nach dem Verdampfen des Alkohols wurde das Prolin in bekannter Weise in das charakteristische Kupfersalz verwandelt, welches in heissem absolutem Alkohol löslich war.

Die Ausbeute an Kupfersalz betrug 1.03 g. Für die Analyse wurde das gereinigte Salz im Vakuum bei 100° getrocknet:

0.1613g Subst. gaben 13.8 c.c. N (22° 752 mm)

	N
$(C_5H_8NO_2)_2Cu$ Ber.	9.60
Gef.	9.59

Ferner wurde das Vorhandensein von Glutaninsäure im Filtrat des phosphowolframsauren Niederschlags durch ihren charakteristischen faden Geschmack ausser Zweifel gestellt. Wegen Mangel an Zeit haben wir diese Säure nicht isoliert.

Aus 4 Kilo Salzbrei des Bonitos wurden isoliert:

1.	Lysin pikrat	18.14g
2.	Histidin pikrat	0.50
3.	Tyrosin	3.00
4.	Leucin	4.06
5.	Alanin	10.80
6.	Leucin+Alanin	4.00
7.	Prolin Kupfer	1.03
8.	Tryptophan	Vorhanden
9.	Arginin di pikrat(?)	1.50
10.	Glutaninsäure	Vorhanden

ON THE CHEMICAL COMPOSITION OF "SAKÉ"

By T. Takahashi and Goro Abe
College of Agriculture, Imperial University, Tokyo, Japan

On the chemical composition of Saké many reports have been made: Some authors write on the composition which is contained originally in the fermented mash and others on the constituents which have been derived from the vat or cask which contain saké. In the factories of saké the fermenting vat and the cask which is employed for the transportation of saké, are made from cryptomeria japonica; and therefore certain components of the material of the vessels must be present and dissolved in saké Ch. Kimoto[1] reported on sughi-oil in 1903 and N. Nagai[2] and T. Kimura made some researches on its chemical composition and found protocatechin, a phinon-like substance, vanillin and a kind of terpene and they have proved the presence of the same substances in saké. The terpene, mentioned by the above authors, was by K. Keimatsu[3] proved to be a sexterpene and he also added as a new component of cryptomeria japonica a phenol-like substance, having reducing property. In the same year Yamamoto and Ishikawa[4] made studies on the same reducing substance as regards its influence in determining the reducing sugar in saké.

On the proper composition of saké K. Keimatsu[5] made a report on the furfurol in saké and one of us[6] made research on the contents of furfurol and found that in young saké, or shortly after the fermented mash is pressed, there was no furfurol or, if

[1]Bulletin of the College of Agriculture Tokyo Imp. Univ. vol. 4. page 403.

[2]In a speech made at the Tokyo Chemical Society (1904).

[3]Yakugakuzasshi (Journal of the Pharmaceutical Society of Japan, 1905, March.)

[4]do: Sept. 1905.

[5]do: Decemb. 1904.

[6]T. Takahashi: The Journal of the Agricult. Society of Japan. May, 1905.

present, it was a trace; while in aged saké, i.e., after the storage during summer, it was always present in it. In the same year H. Nishizaki[7] made a quantitative determination of furfurol, but arrived at somewhat different results from the writer. Recently H. Ito made experiments on this subject[7'] with about 111 samples of saké and arrived at the same conclusion as the writer. One of us[8] has made a report in respect to the quantity of fusel oil and pointed out that the quantity of the substance must be examined when we classify saké as regards quality. Moreover, a small quantity of methyl alcohol was proved to be present in ordinary saké[9], and a somewhat evident quantity of methyl-lactate in certain samples of saké was found by one of us[10].

H. Nishizaki made a report on free acids[11] and sugars[12] of saké and mentioned that the latter consisted chiefly of glucose, while K. Suda[13] already sixteen years ago made experiments on the contents of the sugar of saké and reported that the sugar consists chiefly of maltose. It seems to the writer that both authors may be right; because they used only a limited number of samples and arrived at altogether opposite results. If they had examined a wider range of samples they would probably have found out that their results were one sided. K. Keimatsu[14] and Shimizu reported on the presence of acetaldehyde, fusel oil, succinic acid, lactic acid and acetic acid in saké.

On animo-acids, however, no one has reported yet and we therefore present here the results of our investigations about amino-acids and other components.

The general chemical composition of the samples was as follows:

[7]Journal of the Pharmaceutical Society of Japan. Novemb. 1905.

[7']Journal of Tokyo Chemical Society. Vol. 32, No. 7. He proved directly, chiefly by anillin acetate, but the quantity was too small; he has distilled saké under reduced pressure and below 35°C neutralized saké.

[8]T. Takahashi. Journal of the Agricu. Society of Japan. Apr. 1905.

[9]T. Takahashi. Bulletin of the Agr. Coll. Tokyo Im. Univ. Vol. 6. No. 4.

[10]T. Takahashi. Bulletin of the Agr. Coll. Tokyo Im. Univ. Vol. 7. No. 4.

[11]Journal of the Pharmaceutical Society of Japan. May, 1905.

[12]do: May, 1906.

[13]do: Apr. 1890.

[14]do: Decem. 1905.

1). Total—N	0.1865 %
2). Protein—N.	
a). Stutzer's method	0.0072 %
b). Rumpler's method[15]	0.00672%
c). Precipitate by Pb–acetate and Pb-oxide (Bungener u. Fries. Zeit. f. d. ges. B. 1894.69.)	0.00447%
d). Precipitate by basic lead acetate	0.00435%
3). Non-albuminoid—N.	
a). Ammonia—N. (Wurster's method)	0.00629%
b). Organic base—N	0.0598 %
c). Other—N. (chiefly amino-acids)	0.1131 %
4). Esters (as acetic ester)	0.0457 %
5). Total acid (as succinic acid)	0.2666 %
a). Non-vol. acid (as succinic acid)	0.2596 %
b). Volatile acid (by difference) (as acetic acid)	0.00715%
c). Volatile acid (determined in the distillate) (as acetic acid)	0.0216 %

The protein nitrogen determined by Stutzer's and Rümpler's method is always higher than that of the nitrogen found in the basic-lead-acetate precipitate of lead–acetate and lead oxide; because the former two methods always precipitate in a certain degree a part of albumoses and peptones beside proper proteins. For this reason therefore, we must assume the presence of albumoses and peptones in saké; but we can prove directly the presence of both substances. If we take 100-200 c.c. of saké and after removing the protein by basic lead acetate and from this filtrate after removing lead by H_2S and evaporating to a small volume a sufficient quantity of Zu-sulphate is added to suturaled the solution, acidifying with sulphuric acid, there will be found a precipitate of *albumoses*. From the filtrate of albumoses, after removing zinc by H_2S and condensing in a small

[15]Rümpler: Deutsch. Zeit. Ind. 1898, 1729.

The difference between contents of nitrogen determined by Stutzer's method and that of the precipitate made by lead-acetate is shown from the above table to be 0.0027%, and this must be the least quantity of the nitrogen of albumose and peptones.

volume, this will contain *peptones* in solution, which will be proved easily by Biuret reaction.

(I) Organic Bases

10 litres of the samples were evaporated under reduced pressure and at 60° C. to a small volume, almost equal to $\frac{1}{5}$ of the original volume. After this operation the protein-substance was removed by basic lead-acetate and researches were made about bases according to Kossel's method; but of histidine only a trace was found and the characteristic crystals of the chloride were not obtained, giving Pauly's diazo-reaction (1904). Arginin was not found. Picrate of lysin, about 1.1 grms, was obtained as fine needles and platy crystals, which melted at 230° C. (uncorr.), so that it contained some impurities.

(II) Mono-amino-acids

Ten litres of saké were used for the isolation of mono-amino-acids after E. Fischer's well-known ester method.

a) Under the pressure below 20 m.m.
(Esters prepared from 10 l. saké.)

First fraction	60°–92° C.	1.0 grms.
Second fraction	92°–150° C.	1.5 grms.
Third fraction	150°–235° C.	Trace.

b) Under the pressure below 18 m.m.
(Esters prepared from 10 l. saké.)

First fraction, below	41° C.	0.6 grms.
Second fraction	41°–60° C.	1.0 grms.
Third fraction	60°–98° C.	3.0 grms.
Fourth fraction	98°–150° C.	4.5 grms.

From the first fraction of series (a), 0.2 grms of alanin, 0.2 grms of leucine, and 0.1 grms of prolin were obtained. In the second series 0.2 gr. of alanin from first fraction and 1 g. of leucine, trace prolin from third fraction, were obtained. The Alanin obtained from first series was added to the same from the second series and after purification analysis was carried out. It was very sweet, having a melting point of 243–245° C. (uncorr.)

and decomposed at the point with evolution of gas. The result of analysis was as follows:—

Substance taken:—0.972.
Nitrogen = 12 c.c. (at 15° C. 760 m.m.)
Calculated as $C_3H_7NO_2$ N = 15.67%
Found.....N = 14.51%

Prolin was bitter in taste and its copper salts contained two components, one of which dissolved in absolute alcohol, while others do not, indicating the presence of active and inactive prolin.

Leucin was bitter in slightest degree so that it was washed with absolute alcohol. It gave a melting point of 290–293° C. (uncorr.), changing in brown at 270° C. already. It decomposed at the melting point with emission of gas. The analytical result was as follows:

Substance taken: 0.1226 grms.
Nitrogen.....10.7 c.c. (at 11° C. 758 m.m.)
Substance taken: 0.1102 grms.
Co_2.....0.2165 grms.

Calculated as $C_6H_{13}No_2$	Found.
N = 10.85	10.46
C = 55.80	53.57
H = 10.07	9.73

The above result tells us that the substance was not pure.

Phenylalanin and glutannic acid were not found, but trace of aspartic acid was found.

Leucinimid. ($C_{12}H_2N_2O_2$). After all esters were evaporated, the residue was treated with acetic-ester, which dissolved a part of it. When the dissolved part was evaporated, there remained hexagonal or quadratic plate, which tasted very bitter. The platy crystals were dissolved again in acetic ester and after evaporaton ether and ethyl-alcohol were added, but there were found no crystals. So we could not prove the presence of leucinimid.

Tyrosin. The presence of this substance was easily proved by Millon's reaction by the filtrate from the precipitate of protein—substance of saké. But for the isolation of this substance we followed Brown[16] and Willer's operation, which they employed for the isolation of this substance from malt. Thus 1 grm. of tyrosin was obtained from three litres of saké. While, as in other cases, 10 litres of the sample was evaporated to a small volume and after separation of protein, the filtrate free from lead, was evaporated to almost one-fifth of the original volume of saké and left standing over night in a cool place, there appeared the characteristic silky crystals of tyrosin amounting almost to 6 grams. The isolated tyrosin gave strong red coloration by Millon's reagent, faint reaction of Pauly's[18] diazo-reaction strong by Wurster's reaction[17] and Denige's reaction.[19] The analytical result was:

Substance taken: 0.1048 grms.
Nitrogen.....6.8 c.c. (at 10° C. 762 m.m.)
Calculated as $C_9H_{11}NO_3$..... N=7.777%
Found..... N=7.79%

Cystin. This substance was proved very easily in the filtrate which was obtained in removing the protein-substance of saké, but the quantity was too small to isolate it.

Tryptophane, ($C_{11}H_{12}N_2O_2$). This substance was obtained from 110 c.c. of saké by Hopkins' and Cole's[20] method. The crystals were platy and bright; giving red coloration with bromine-water and precipitated by phosphotungstic acid. A few crystals were mixed with caustic potash and after fusing and subjected to the dry distillation, pyroll reaction was observed in this distillate. However, the presence of tryptophane in saké is limited only to young saké, or not aged saké.

[16] Brown u Willer. Woch. f. Brauerei, 1907. Nr. 11. S. 139.

[17] Beautiful red color by acetic acid and natrium-nitrite (c. f. Ph. 1. 1903 (1888).

[18] Hoppe-Seyler's zeit. f. ph. ch. 42, 517 (1904).

[19] Wine-red by form aldehyd and H_2SO_4 (comptes rendus, 130, 583, 1900).

[20] Journal of Phys. 27, 418 (1902), 29, 451 (1903).

On this fact one of us[21] has reported already. On the question, that why this substance disappears in the aging process of saké, H. Ito[22] has made some observations and arrived at the conclusion that the tryptophane in young saké is assimilated or rather decomposed by so-called aging yeast[23]:—Willia anomala varieties.

The Substances which dissolved in Ether

1.) Succinic acid.

Ten litres of saké were evaporated to about 400 c.c. under reduced pressure below 70° C. The syrup thus obtained was extracted with ether, using Kumazama and Sudo's extraction apparatus and dried. From ether extract, the ether was evaporated for a long time in sulphuric acid containing desiccator, then there appeared long platy or mono-crinic prismatic crystals in a brown colored syrup. The crystals were separated by filtration and after washing very quickly with a small quantity of cold absolute alcohol, there remained rather small quantities of the crystals, which melted at 181°–182.5° C. (uncorr.). The substance behaves very strongly acidic to litmus, tasting characteristic to succinic acid, and gave pyroll reaction when subjected to Neuberg's prove (1900–1901). Such pure substance obtained from ten litres of saké was about 3 grams.[24]

From another 18 litres, 4.7 grams of the substance was obtained as raw product, which was brown in color, so that it was first neutralized with a 5% solution of natrium hydroxide, and after evaporating to syrup, absolute alcohol was poured on there appeared almost spontaneously fine crystals of natrium succinate. The natrium salt thus obtained was washed repeatedly with absolute alcohol. It was silky white and gave a brown precipitate with ferric chloride.

[21] T. Takahashi. Journal of the Tokyo Chemical Society of Japan, Vol. 32, No. 3, 1911. Also, Journal of the College of Agr. Tokyo Imp. Univ. Vol. 000, No. 0.

[22] H. Ito. Journal of the Tokyo Che. Soc. of Japan, Vol. 32. No. 7, 1911. Also, Journal of the College Agr. Tokyo Imp. Univ. Vol. 00, No. 00.

[23] T. Takahashi's report. Journal of the College of Agr. Tokyo Imp. Univ Vol. 1, No. 3.

[24] The inactive form of zinc salt contains 18.17% of water of crystallization.

2). Lactic acid.

The syrup obtained after removal of succinic acid crystals was neutralized with zn-carbonate to attain a thick pasty mass from which, after standing overnight, the crystals of zinc lactate were separated by "nutsche" and washed with absolute alcohol. The salt thus obtained was treated with H_2S to separate zinc, and the colorless solution of free acid thus obtained gave intense reaction of Ueffermann's proof. Zinc salt was reprepared from this pure lactic acid and after drying well in the desiccator, the water of crystallization was determined, drying several hours at 105–110° C. The water[25] of crystallization amounted to 17.0%, almost equal to that of the inactive form of lactic acid.

3). Tyrosol. $OHC_6H_5CH_2CH_2OH$.

For the isolation of tyrosol we followed Fe. Ehrlichi's[26] method. Ten litres of the sample were evaporated to almost 400 c.c. and after basifying with $NaHCO^3$, ether extract was made by Sudo and Kumagawa's extraction apparatus. The yield was 4 grams as raw product. It was dissolved again in absolute alcohol and decolorized with animal black. It behaved very strongly to Millon's reagent and diazobenzol sulfonic acid and faintly to ferric chloride and Denigè's–Moerner's proof. Tyrosol-di-benzoate prepared by the addition of benzoyl chloride and natroulye, melted at 113° C. (uncorr.).

Summaries

The above statements are summarized below:

The yield of the observed substance from 10 litres of Saké:—

1). Glycocoll..... Not found.
2). Alanin..... 0.2 grams.
3). Leucin..... 0.6 grams.
4). Prolin (active and racenic)..... 0.1 grams.
5). Phenylalanin..... Not found.

[25] Ber. I. Deut. ch. G XLIV, Heft I. S. 139–146, 1911, u Bioch. Zeit, 36 Band, 15 Heft. S. 477, 1911.

[26] F. Ehrlich, Ber. d. Deut. ch. Ges. 1911, XLIV, Heft I. S. 143.

6). Glutamic acid ..... Not found.
7). Aspartic acid ..... Trace (?)
8). Leucin-imid ..... Not found.
9). Tyrosin ..... 6.0 grams.
10). Cystin ..... Trace.
11). Tryptophane (only present in young saké) ..... 1.0 grams.
12). Lysin ..... 0.25 grams.
13). Ristidin ..... Trace.
14). Arginin ..... Not found.
15). Tyrosol ..... 4 grams (raw products).
16). Succinic acid ..... 3 grams (in the least).
17). Lactic acid (inactive) ..... 2 grams (in the least).
18). Albumoses and peptones ..... present.

The yield of amino-acid is too small in comparison to the nitrogen contents of amino-acids obtained from calculation. The main cause is that the pressure, under which we have made fractions was rather high (18–20 mm.), but beside this saké contains rather high percentage of carbohydrate, which makes it difficult to isolate the esters. In conclusion the writer must offer many thanks to his assistant M. Sato, for his faithful help during this research.

(*Abstract*)

A STUDY OF THE COMPOSITION OF CIDER VINEGAR

MADE BY THE GENERATOR PROCESS

L. M. Tolman and E. H. Goodnow

Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.

This paper is the result of factory experiments on a very large scale of the conversion of fermented cider into vinegar in the ordinary commercial type of vinegar generator.

Experiments were carried on for a period of several months, and some 40,000 to 50,000 gallons of hard cider were run through a series of generators, samples being taken from the material going on to the generator and from the finished product, and analyses made in detail to show the changes which took place. Work was undertaken largely because the existing data published, regarding American cider vinegars was based wholly upon the analyses of vinegars made in a small way, and fermented under the slow fermentation process, which takes place in a barrel or cask. It was found at the beginning of this investigation that the results of analyses on this type of vinegar showed such a wide variation that it was practically impossible to detect any forms of adulteration. And it was concluded from our analyses of ciders existing at that time that this wide difference in results was due to the method of manufacture; and as the method of manufacture in this country at the present time is practically confined to the generator process, it was found that it was necessary to have data upon this product.

Results of the investigation showed that vinegars made by the generator process were practically as uniform in composition as the cider from which they were made, showing a very different condition from that found to exist in the existing data on cider vinegar.

THE MICROSCOPICAL EXAMINATION OF VEGETABLE PRODUCTS AS AN ADJUNCT TO THEIR CHEMICAL ANALYSIS

By A. L. Winton

U. S. Food and Drug Inspection Laboratory, Chicago, Ill.

In solving the problems of man and nature the analytical chemist too often limits himself to chemical or physico-chemical methods. He is an analytical chemist in the strict sense of the word and not an analyst, which implies a man of broader training and experience, utilizing the principles of other sciences as means to his end. He turns his back on the methods of vegetable and animal histology, physiology and bacteriology, asserting with satisfaction that he is a specialist and as such must limit his field of activity.

This attitude of the analytical chemist may be traced to a misapprehension as to the province of a specialist. Such a worker must be limited only in the field of application and not in training or the methods employed. An occulist, for example, limits himself to defects of vision and diseases of the eye and allied organs, but in order to properly carry out the work of his specialty he must have broad medical training and be conversant with the general principles of optics, bacteriology, chemistry and perhaps other sciences. Specialists in other sciences, both pure and applied, must also have good general training if they are to achieve distinction in their limited fields; otherwise they are in much the same position as the mechanic who, instead of mastering his trade, learns to operate one machine, thus becoming a mere automaton.

Botany and chemistry are generally considered incompatibles. The student of chemistry sometimes takes up bacteriology as a minor subject, but comparatively seldom studies advanced botany, even though he intends to specialize in food analysis, textile chemistry, paper technology or some other subject dealing chiefly

with materials of vegetable origin. No physiological chemist would think of pursuing his investigation of animal materials without a working knowledge of animal anatomy, yet agricultural and food analysts and others dealing with vegetable materials too often limit themselves to a knowledge of chemical constituents, ignoring the relation of composition to histological structure.

This is most remarkable, since the methods of vegetable histology, as well as of chemistry, are invaluable in solving problems relating to the nature or constituents of foods, drugs, fibers and other products of vegetable origin. Sometimes one line of investigation alone is useful, sometimes the other, but often each throws some light on the subject, and the corroboratory results obtained by such widely differing means furnish an indisputable chain of evidence.

Let us look more closely into the nature and relation of these two applied analytical sciences.

Chemical analysis deals with chemical constituents; microscopical analysis deals largely with the form of some of these constituents. Chemical analysis determines the amount of fiber, starch, protein, oil, etc.; microscopical analysis determines the shape, size, and other characteristics of the cells and cell contents. Chemical analysis usually stops with the mere determination of the amount of chemical constituents; microscopical analysis goes further and names the particular product from which they were derived. Chemical analysis answers a question only in scientific terms; microscopical analysis, in terms which all can understand.

In many cases, the best idea of a material is gained by following out both lines of investigation. By chemical analysis we learn the percentage of protein, fiber, starch, etc., but not the ingredients from which they were derved; by microscopical analysis we learn the ingredients, but usually gain only an approximate idea of their proportion. Given the results of both analyses, we may often calculate with some exactness the percentage of the different materials present.

If, for example, we find in a sample of wheat bran 11 instead of 16 per cent. of protein, and 15 instead of 8 per cent. of fiber, we know it is not pure bran but we do not know the adulterant;

if we find corn-cob tissues under the microscope, we learn the adulterant but not the amount. Knowing that the material is a mixture of bran and ground corn-cob, and knowing the average percentage of protein and fiber in both, we are in a position to calculate from the results of the chemical analysis the relative amounts of these ingredients.

Again, if we find in ground mace 40 per cent. instead of 20 per cent. of fixed oil, we know it is not pure mace; if we find under the microscope a large amount of tissues of the Bombay mace, a material worthless as a spice containing about 60 per cent. of fixed oil, we learn the adulterant. Knowing all this, and knowing the average percentage of oil in true mace and Bombay mace, we have the data for calculating roughly the percentage of each in the mixture.

Still again, if in a textile fabric we find a certain percentage of organic fiber insoluble in boiling alkali, we know that the fabric is not all wool. If under the microscope we identify this insoluble fiber as cotton, we have found the missing link in the chain of evidence.

In the analysis of complicated mixtures, we must often rely entirely on microscopical examination. For example, chemical analysis of a mixture of wheat, buckwheat and corn flours gives us little information, and it is only after the characteristic starch granules and tissues of each have been found under the microscope that we gain a definite idea of the nature of the constituents.

Again, in the examination of paper, the microscope is our sole dependence in learning the nature and approximate percentages of the fibers employed, chemical analysis serving merely to determine the kind and amount of sizing, coating and other non-fibrous constituents.

Among some condimental cattle foods examined by the writer some time since was one, the chemical analysis of which disclosed but one proximate constituent, viz., common salt; the microscope, however, disclosed linseed meal, corn meal, wheat feed, mustard hulls, cocoa shells, malt sprouts, fenugreek and turmeric. In such a case, dependence must be placed entirely on the microscope, except for mineral ingredients.

Chemical analysis of another sample demonstrated the presence

of ground bone, carbonate of lime, iron oxide and free sulphur; microscopical examination disclosed linseed meal, wheat feed and charcoal. This is a striking example of a material in which half the constituents (all mineral) can only be detected by chemical analysis; the other half (all vegetable) by the microscope.

Many other equally striking examples of the interdependence of these two applied analytical sciences might be cited.

The point now arises as to who is to carry on these two lines of investigation so different in details but so similar in purpose.

One plan is for a chemist to make the chemical analysis and a botanist the microscopical examination. This plan has the advantage that each can confine his attention to one specialty, but it had the disadvantage that the close partnership between the two, which is essential to the best results, outside of large institutions, is both difficult and expensive. Such a division of labor would usually be as impracticable as to divide the work of a chemical laboratory between a chemist and a physicist, the former conducting the precipitations and other chemical processes the latter, polarizations, determinations of specific gravity, refractive index and the like.

The rational plan is for one man to master both lines of research. Such a man need not execute all the details, but he should be thoroughly acquainted with them and should interpret the results. We will call him an analyst, not a chemist or a botanist, and his laboratory an analytical laboratory, not a chemical or botanical laboratory. His equipment should consist of the necessary apparatus for a wide variety of chemical work and a complete microscopical outfit, including micro-reagents and a set of standard specimens of economic seeds, roots, barks, fibers, woods, etc.

But in order to have workers in this field, we must have suitable courses of instruction in our schools of science. The subject has a recognized place in many continental universities, particularly in the schools of medicine, pharmacy and hygiene, but outside of a few institutions, receives little attention in America.

The student who seeks to prepare himself for this field should take both chemical and botanical studies. In chemistry, he

should study the branches taught in a well-regulated chemical course—elementary chemistry, qualitative and quantitative analysis, organic and physical chemistry, and so on. In botany he should take up successively elementary botany, systematic botany (at least of the phanerogams) and vegetable anatomy and physiology. These studies are all on the curriculum of every college and school of technology, although the student of chemistry does not usually take all the botanical studies named. Without a certain amount of botanical training, however, a chemist is no more fitted to take up microscopical analysis than a botanist without chemical training is fitted to work at quantitative analysis.

After his preliminary studies in chemistry and botany, the student is ready to take up a course in the methods for the chemical and microscopical examination of the various raw materials and of the products derived from them. This course should be so arranged that the student will carry along his chemical and histological practice side by side, as he must do afterwards in practical work. For example, in studying the cereal grains, he should devote part of his time to the methods of determining water, ash (including ash analysis), protein, fiber, starch, fat, pantosans, etc., and another part to a systematic study of the starches and the histological elements of the bran coats both in sections and in powdered form. In like manner, he should take up a chemical and histological study of leguminous seeds, oil seeds, spices, tea, coffee, cocoa, drugs, fibers, etc.

His work in the chemical laboratory should teach him not only the strictly chemical methods but also the use of the polariscope, the spectroscope and other physical apparatus, and his microscopical instruction should fit him not only to differentiate organized forms but other characteristic elements, such as fat crystals, mineral crystals, and the like.

After such a course, he should be able not only to undertake investigations in physiological or plant chemistry but also the laboratory work of an official food department or a custom house, a flour mill, a brewery, a sugar refinery, a candy works, a fruit cannery, a drug mill, a textile mill, a paper mill, etc.

It is my firm belief that courses similar to that outlined should

be conducted in all our leading universities and schools of technology, and the student should be taught the use of the microscope in conjunction with the balance in solving the analytical problems which every day become more numerous and intricate.

GENERAL INDEX
TO THE TWENTY-FOUR VOLUMES OF ORIGINAL COMMUNICATIONS

Volume	Section	
1	I.	Analytical Chemistry.
2	II	Inorganic Chemistry.
3	IIIa	Metallurgy and Mining.
4	IIIb	Explosives.
5	IIIc	Silicate Industries.
6	IV	Organic Chemistry.
7	IVa	Coal Tar Colors and Dyestuffs.
8	Va	Industry and Chemistry of Sugar.
9	Vb	India Rubber and other Plastics.
10	Vc	Fuels and Asphalt.
11	Vd	Fats, Fatty Oils and Soaps.
12	Ve	Paints, Drying Oils and Varnishes.
13	VIa	Starch, Cellulose and Paper.
14	VIb	Fermentation.
15	VII	Agricultural Chemistry.
16	VIIIa	Hygiene.
17	VIIIb	Pharmaceutical Chemistry.
18	VIIIc	Bromatology.
19	VIIId	Biochemistry including Pharmacology.
20	IX	Photochemistry.
21	Xa	Electrochemistry.
22	Xb	Physical Chemistry.
23	XIa	Law and Legislation Affecting Chemical Industry.
24	XIb	Political Economy and Conservation of Natural Resources.