THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

An Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, May 6th, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—Raymond Merefield Edwards, B.Sc., Llewelyn John Howells, B.Sc., Donald Neil McArthur, D.Sc., Ph.D., F.I.C., F.R.S.E., James Sword, M.A., B.Sc., Ph.D., A.I.C.

Certificates were read for the second time in favour of:—George Brown, A.I.C., Charles Louden, B.Sc., A.I.C., Charles Percy Money, B.Sc., F.I.C., Martin Priest, F.I.C., Arthur Goodyear Simpson, M.A., Gerrish Smith.

The following were elected Members of the Society:—K. N. Bagchi, B.Sc., M.B., D.T.M., William Nelson Bradshaw, B.Sc., Adrian Joseph Clifford Lickorish, F.I.C., Ernest Grenville Purser, B.Sc., A.I.C., and William Waddell Robson.

The following papers were read and discussed:—"A Demonstration of a New Development in Filter Papers," by E. J. Guild; "The 'Rope' Spore Content of Flour and its Significance," by A. J. Amos, B.Sc., A.I.C., and D. W. Kent-Jones, Ph.D., B.Sc., F.I.C.; "The Separation of Tin from Tantalum and Niobium," by W. R. Schoeller, Ph.D., and H. W. Webb (*Work done under the Analytical Investigation Scheme*); "A New Method for Detecting Decomposition Products in Anaesthetic Chloroform," by N. L. Allport, A.I.C.; and "Contaminations in Morphine Deposited in the "British Pharmacopoeia" Process for the Analysis of Opium," by J. N. Rakshit, F.I.C.

Obituary.

MEREDITH WYNTER BLYTH.

Meredith Wynter Blyth was born at Worcester on December 28th, 1871, and died at Tankersley, near Barnsley, on March 31st, 1931.

He was the son of Dr. Wynter Blyth, an original member of the Society, whose well-known works, "Poisons, their Effect and Detection," and "Foods, their Composition and Analysis," he revised and largely rewrote.

He was educated at King's College School and St. John's College, Cambridge, where he gained a double first in the Natural Science Tripos. After leaving Cambridge, he took his Science Degree at London University, and became Gas Examiner for the London County Council, later working as assistant to Klein, at St. Bartholomew's Hospital.

He joined the Society of Public Analysts in 1902, and served on the Council in 1906–7. His contributions to THE ANALYST included papers on the detection of preservatives and colouring matters in milk, and on the chemical control and standardisation of disinfectants. In 1896 he was elected an Associate of the Institute of Chemistry, and became a Fellow in 1899.

In 1898 Wynter Blyth started in private practice as a consulting chemist and bacteriologist, and for over 20 years was Public Analyst for the boroughs of Brighton and Eastbourne. In 1905 he joined the staff of Newton, Chambers & Co., Ltd., near Sheffield, as Chief Chemist. He carried out much of the early work on the bacteriological standardisation of disinfectants, and he contributed very largely towards raising their reputation to that in which they are held to-day.

Wynter Blyth was a man brilliant in argument, both in speech and in writing. He had a very keen sense of humour and a ready tongue, and his kindness of heart, his sociability, and his prowess at games, which commenced by his representing his college at lawn tennis with the Doherty brothers, made him universally popular.

W. NEWTON DREW.

The Rapid Determination of Solid Saturated Fatty Acids.

BY T. P. HILDITCH, D.Sc., F.I.C., AND J. PRIESTMAN, PH.D.

(Work done under the Analytical Investigation Scheme.)

(Read at the Meeting of the North of England Section, February 14, 1931.)

WE have had under consideration for some time the methods available for determining rapidly the amount of saturated fatty acids in the mixed fatty acids of natural fats, and, in connection with this subject, the Analytical Investigation Committee of the Society suggested that, possibly, an accurate measure of the "solid" unsaturated acids in hydrogenated fats might be obtained by a combination of the lead salt (Twitchell) separation with an oxidation method such as that of Bertram. Subsequently, however, we learned that Mr. L. V. Cocks and his colleagues, in the Research Laboratories of Messrs. Lever Brothers, Ltd., were making a comprehensive investigation of modifications in the Twitchell lead salt separation which

should permit of the determination of "solid" unsaturated (iso-oleic) acids as well as of saturated fatty acids, and we accordingly confined our attention mainly to the determination of saturated acids in non-hydrogenated fats. During the later stages of the work the results obtained by Mr. Cocks and his colleagues and by ourselves have been mutually discussed from time to time. The respective investigations have, of course, been undertaken quite independently by each group, working under different auspices, and to a minor extent, from not quite the same standpoint; but the researches are complementary, and it is, therefore, appropriate that the results should be presented concurrently, and considered in conjunction with each other.

The methods available for the estimation of saturated higher fatty acids depend either on the relative insolubility in different solvents of certain of their metallic salts, or on removal of all unsaturated acids by oxidation. It is proposed to review each of these classes of procedure, but, at the outset, we would point out that no existing method is applicable to the whole range of fatty acid mixtures found in natural fats, and that the type of fat under examination must be considered in every case. Furthermore, it is desirable to consider the probable limits of experimental error liable to be encountered. From the descriptions of some workers it would appear that results can be duplicated and an accurate estimate given for the content of saturated acids to within a few tenths per cent.; in our opinion, in the majority of cases, the order of experimental error is more probably in the neighbourhood of $\pm 1{\cdot}0$ unit per 100 parts of total mixed fatty acids, whilst in cases in which saturated acids containing 14 carbon atoms or less per molecule or certain unsaturated acids such as erucic, petroselinic or other solid oleic acids are present, an accuracy of ± 2–3 units per cent. is the most that can be expected.

In the course of our work we have considered both the lead salt (alcohol) separation and the oxidation processes, and these will be dealt with consecutively.

Separation of Saturated Fatty Acids as Metallic (Lead) Salts.—This method has, of course, been available since Gusserow (*Annalen*, **1828**, **27**, 153) and Varrentrapp (*ibid.*, 1840, **35**, 196) drew attention to the differing solubility of the lead salts of the higher saturated and unsaturated acids in ether. Many modifications of their original process have been proposed,* some of which advocate the replacement of ether by other organic solvents, whilst others have suggested the use of salts of univalent metals such as lithium or thallium. The employment of a univalent metal would be expected to promote separation of saturated from unsaturated acids owing to the impossibility of obtaining mixed salts containing saturated and unsaturated acids in combination with one and the same basic group; but the general experience has been that, even with the thallous salts proposed by Meigen and Neuberger (*Chem. Umschau*, 1922, **29**, 342) and by Holde, Selim and Bleyberg (*Z. deutsch. Oel u. Fett-Ind.*, 1924, **44**, 277, 298), the separation is not more satisfactory than with lead salts. Presumably, the solubility

* A bibliography is given by Bertram, *Z. deutsch. Oel u. Fett-Ind.*, 1925, **45**, 733.

relationships of such salts of oleic acid and the related saturated acids are too closely alike for separation to be effective in these cases.

It is generally agreed, at all events, that the separation of the lead salts from solution in 95 per cent. alcohol at about 15° C. on the lines recommended by Twitchell (*J. Ind. Eng. Chem.*, 1921, **13**, 806) gives the best results. This author recommends adding, to a solution of mixed fatty acids containing 1 to 1·5 grm. of saturated acids dissolved in boiling 95 per cent. alcohol (30 ml.), a solution of lead acetate (about 1·5 grm.) in boiling 95 per cent. alcohol (70 ml.), cooling the mixed solutions slowly to 15° C., and leaving them over-night; the separated lead salts are filtered off, washed with 95 per cent. alcohol until, on dilution, the washings remain clear, and are then recrystallised from 95 per cent. alcohol (100 ml., containing 0·5 grm. glacial acetic acid). The cooling process is repeated, and eventually the separated, washed lead salts are converted by acidification with nitric acid into the free acids, in which form they are weighed.

The proportion of alcohol employed in the first precipitation per unit weight of mixed fatty acids may thus vary, according to the expected percentage of saturated acids in the latter, from about 10 to 30; for mixed fatty acids containing about 30 per cent. of saturated acids it is about 20. Again, whilst Twitchell mentioned the use of sufficient lead acetate to interact with all the fatty acids present, he was inclined to recommend that the lead acetate should be taken so as to be only somewhat in excess of that required for combination with the saturated acids; later workers have reverted to some extent to the use of larger proportions of lead acetate, and in a recent communication describing the application of the Twitchell method to directly saponified fats Baughman and Jamieson (*Oil and Fat Ind.*, 1930, **7**, 331) advocate the employment of 5 grms. of lead acetate per 1 to 1·5 grm. of solid acids present in the original fat under test.

The important point to be considered is the extent to which saturated acids may pass into the soluble lead salt fraction and, during a somewhat extensive experience of a modified form of the Twitchell method as a preliminary to fractional distillation of the methyl esters of the "solid" and "liquid" acids so obtained, we have formed the opinion that appreciable quantities of myristic, and still more of lower, acids may find their way into the soluble lead salts or "liquid" acid portion. Further, but to a much less degree, lead palmitate and even lead stearate may, in particular cases, be found with the unsaturated acids in the soluble lead salt fraction.

This may be illustrated by typical examples from the numerous detailed analyses of natural fatty acid mixtures in our laboratory records (which have, with a few exceptions, already been published in various communications). For the fractionation analysis, it is found most suitable to employ, per unit of mixed fatty acids, 10 parts of 95 per cent. alcohol and 0·7 part of lead acetate, and, in some cases, the first crop of "solid" acids has been submitted to a repetition of the lead separation. Table I shows the respective proportions of lauric and lower acids

("C_{12} and below"), myristic ("C_{14}"), palmitic ("C_{16}"), stearic ("C_{18}"), palmitoleic or lower unsaturated acids ("lower unsaturated"), oleic and linoleic ("C_{18} unsaturated"), C_{20} or C_{22} unsaturated acids ("higher unsaturated") and unsaponifiable matter estimated by fractionation analysis in the "solid" (S) and "liquid" (L) acid groups.

TABLE I.

Mixed acids of:		C_{12} and below.	C_{14}.	C_{16}.	C_{18}.	Lower unsaturated.	C_{18} unsaturated.	Higher unsaturated.	Unsap.
Butter* (*a*)	S	1·0	8·5	26·1	6·5	—	12·8	—	—
	L	5·9	1·9	—	—	—	32·2	—	0·4
Butter* (*b*)	S	0·9	6·3	27·3	11·5	—	5·9	—	—
	L	7·0	4·8	—	—	—	29·9	—	0·3
Sperm head oil	S	11·1	11·5	8·0	1·7	9·7	8·3	1·5	0·5
	L	8·3	2·0	0·3	—	23·5	8·4	5·0	0·2
Laurel fat	S	23·5	—	8·8	—	—	15·4	—	3·0
	L	8·2	—	—	—	—	34·5	—	6·6
Nutmeg butter	S	1·0	60·1	8·2	—	—	1·7	—	—
	L	0·2	1·5	—	—	—	7·8	—	19·5
Palm oil (*a*)	S	—	0·3	39·7	4·3	—	2·1	—	—
	L	—	0·9	3·1	0·1	—	49·2	—	0·3
Palm oil (*b*)	S	—	1·5	40·1	3·5	—	2·4	—	—
	L	—	0·9	0·7	—	—	50·7	—	0·2
Stillingia tallow	S	—	1·7	62·8	1·2	—	0·5	—	—
	L	1·9	2·0	3·4	—	—	26·4	—	0·1
Cacao butter	S	—	—	21·0	34·4	—	2·1	—	—
	L	—	—	4·7	—	—	37·6	—	0·2
Borneo tallow	S	—	—	18·5	36·8	—	1·9	—	0·2
	L	—	1·4	2·7	2·2	—	35·7	—	0·6
Mutton tallow	S	—	0·1	24·1	30·5	—	7·6	—	—
	L	—	4·5	0·5	—	—	32·7	—	—
Beef tallow (*a*)	S	—	0·4	30·6	19·1	—	3·9	—	—
	L	—	4·0	—	—	—	41·8	—	0·2
Beef tallow (*b*)	S	—	1·2	27·3	14·1	—	3·9	—	—
	L	—	5·1	0·1	—	—	48·0	—	0·3
Olive oil	S	—	—	9·6	1·9	—	4·4	—	—
	L	—	1·1	—	—	—	82·2	—	0·8
Cod-liver oil, Scottish	S	—	2·5	9·9	—	4·5	10·2	19·4	—
	L	—	1·0	0·3	—	11·3	14·3	25·6	1·0
Cod-liver oil, Newfoundland	S	—	3·0	7·3	0·6	1·2	4·4	4·6	—
	L	—	2·8	1·1	—	19·0	24·7	30·4	0·9

* Analyses on mixed fatty acids after removal of volatile acids by prolonged steam-distillation (*cf.* Hilditch and Sleightholme, *Biochem. J.*, 1930, **24**, 1098).

Since, for the purpose of fractionation analyses, the quantities of acids submitted to the lead salt separation are of the order of 100–200 grms., it is not to be expected that the latter can be regulated so exactly as in the actual Twitchell analysis on about 5 grms. of mixed acids; nor is this essential for the purpose of ester analysis by the fractionation method. Apart from this, however, Table I

demonstrates that, when using a ratio of fatty acid: alcohol of 1:10, saturated acids may pass into the soluble lead salt portion somewhat as follows:—

(i) Stearic acid is practically always absent from the "liquid" acids.

(ii) Palmitic acid is usually absent or only present in traces in the "liquid" acids, but has been occasionally observed in more appreciable amounts; this is usually associated with a fatty acid mixture (cacao butter Borneo tallow, etc.) rich in palmitic and stearic acid, but with a comparatively low oleic acid content.

(iii) Myristic acid usually passes to a considerable extent into the soluble lead salts. The solubility of lead myristate in the alcoholic solution of the soluble lead salts appears to be affected by the composition of the particular mixture of fatty acids present.

(iv) Saturated acids lower than myristic, of course, give lead salts which are increasingly soluble in 95 per cent. alcohol.

In order to throw further light on the above data we have made some tests of the analytical lead salt separation, as described by Twitchell (*loc. cit.*), on the mixed acids of a beef tallow, of olive oil, a Newfoundland cod-liver oil, cottonseed oil and palm oil, mainly employing 1·5 grm. of lead acetate per 1–1·5 grm. of saturated fatty acids.

The composition of the fatty acids in each of these oils had been previously estimated by other workers in this laboratory by the fractionation method (with, we believe, a general order of accuracy of within 1 unit per cent., except in the case of the unsaturated acids of cod-liver oil) with the following results:—

TABLE II.

Acids of:	Beef tallow.	Olive oil.	Cod-liver oil.	Cottonseed oil.	Palm oil.
Saturated:					
Myristic	4·5	1·1	5·8	3·3	2·5
Palmitic	30·6	9·6	8·4	19·8	40·8
Stearic	19·0	1·0	0·6	1·3	3·5
Arachidic	0·1	0·9	—	0·6	—
Unsaturated:					
Below C_{18}	—	—	20·2	—	—
C_{18}	45·6	86·6	29·1	74·2	53·0
Above C_{18}	—	—	35·0	—	—
Unsaponifiable	0·2	0·8	0·9	0·8	0·2
Saturated acids per cent. ..	54·2	12·6	14·8	25·0	46·8

The results of typical determinations by Twitchell's method, as given in his original paper and summarised on p. 356 of this communication, are given in Table III. Slight modifications in the various experiments are indicated by the appended footnotes. The weight of solid acids obtained was corrected for unsaturated acids on the assumption that the observed iodine values were due only to oleic acid; reference to Table I will show that a considerable proportion of the "solid" unsaturated acids from cod-liver oil is made up of acids of the C_{20} and C_{22} series, so that in this instance the correction is only approximately valid.

TABLE III.

Mixed fatty acids. Grms.	Acids from insoluble lead salts. Grm.	Iodine value.	Per Cent.	Saturated acids. Per Cent.
		(i) Beef tallow.		
(*a*) 3·174	1·597	3·8	50·3	48·2
(*a*) 2·521	1·255	4·2	49·8	47·5
(*a*) 3·004	1·520	4·8	50·6	47·9
(*b*) 2·817	1·590	9·5	56·4	50·4
(*b*), (*e*) 2·784	1·445	7·7	51·9	47·5
		(ii) Olive oil.		
(*a*) 5·560	1·050	37·4	18·9	11·0
(*a*) 5·945	0·833	17·1	14·0	11·3
(*b*), (*c*) 10·025	1·138	2·2	11·35	11·1
(*b*), (*d*) 4·933	0·615	8·4	12·5	11·3
(*b*), (*e*) 4·837	0·545	10·8	11·4	10·0
		(iii) Cod-liver oil (Newfoundland).		
(*a*) 6·298	1·070	27·5	17·0	11·8
(*a*) 5·476	0·785	10·5	14·3	12·6
		(iv) Cottonseed oil.		
(*b*) 4·562	1·066	2·8	23·4	22·7
(*b*) 5·261	1·214	3·0	23·1	22·3
(*b*), (*e*) 4·315	0·975	8·4	22·2	20·1
		(v) Palm oil.		
(*b*) 2·973	1·381	2·9	46·5	45·1
(*b*) 2·743	1·269	1·7	46·2	45·3
(*b*), (*e*) 2·652	1·204	3·0	45·4	43·9
(*b*), (*e*) 2·865	1·295	1·8	45·2	44·3

(*a*) Alcohol solutions cooled on bench overnight; temperature not likely to have fallen below 12–15°C.

(*b*) Alcohol solutions cooled in cupboard overnight and minimum temperature observed to be above 15° C.

(*c*) Ten grms. olive oil, *i.e.* ca. 1·2 grm. saturated acids, with 1·5 grm. lead acetate.

(*d*) Five grms. olive oil with 0·7 grm. lead acetate.

(*e*) Lead acetate equal to weight of total acids, *i.e.* 140 per cent. of theoretical for combination with all the acids.

These figures confirm the view that Twitchell's method, as originally described, frequently gives rather low values for the content of saturated acids, whilst the iodine values of the separated acids may be high when the original mixed acids contain a preponderance of unsaturated acids (this is not necessarily a detriment so long as absence of linoleic acid from the separated "solid" acids is assured).

The employment of lead acetate in amount equal to the whole of the mixed acids seems, however, not to improve the separation and, indeed, definitely to impair it when the total content of saturated acids is low (*e.g.* below 25 per cent.).

It is essential that the temperature of the alcoholic solution should not fall below 15–16° C. during standing; exposure to a lower temperature leads to risk of still lower results than would otherwise be obtained. This may possibly have operated to some extent in some of the analyses marked *a* in Table III.

Our conclusions as regards the lead salt alcohol separation may be summarised as follows:

(i) The use of insufficient lead acetate for union with all the fatty acids present, as originally proposed by Twitchell, may lead, in certain cases, to results of doubtful analytical value, although the data obtained for the saturated acid content of mixtures of palmitic and stearic (or higher saturated) acids with oleic, linoleic, linolenic or palmitoleic acids are usually within 1–2 units of the true figure, and are sufficiently accurate for many purposes.

(ii Employment of greater proportions (*e.g.* an amount equal to that of the total fatty acids taken) of lead acetate than those prescribed by Twitchell, leads to lower rather than to higher values for the fully-saturated acids, and cannot be recommended.

(iii) If the mixed fatty acids contain, in addition to the foregoing, more than 3 per cent. of myristic acid, the value obtained will commence to be appreciably lower than the true figure.

(iv) The saturated fatty acids of fats containing notable amounts of lauric or lower acids (such as the nut oils, butter-fats, etc.) cannot be satisfactorily analysed by the lead salt separation method.

(v) The saturated fatty acids of fats containing, as well as oleic acid, acids of the oleic series with 20 or more carbon atoms per molecule (fish oils, oils of the *Cruciferae* and *Umbelliferae*, etc.) cannot be satisfactorily analysed by the lead salt separation method.

(vi) Owing to small amounts of saturated acids passing into the "liquid" acids, even in the case of mixtures containing only palmitic and stearic acids, and still more so when myristic acid is also present, the iodine value of the "liquid" acids is somewhat lower than that due solely to the oleic, linoleic, etc., acids present. If the proportion of linoleic acid to oleic acid is not great, the error introduced by estimating this proportion from the observed iodine value of the "liquid" acids becomes disproportionately large. In any case, we do not recommend the estimation of the proportions of oleic and linoleic acids in a mixture of these two acids by means of the iodine value of the "liquid" acids from the lead salt separation.

(vii) The temperature of the alcoholic solution of lead salts must not be allowed to fall below 15–16° C.

(viii) Subject to the limitations set out above, there are many oils, vegetable and animal, which contain palmitic, stearic, oleic and linoleic acids with little or no myristic or other acid to which the lead salt separation, in its most effective form, can be usefully applied in the approximate determination (to within 1 to 2 units per cent.) of saturated higher fatty acids.

SEPARATION OF SATURATED FATTY ACIDS AFTER REMOVAL OF UNSATURATED ACIDS BY OXIDATION.—According to Bertram (*Z. deutsch. Oel und Fett-Ind.*, 1925,

45, 733) the alkaline solution (200 ml. from an original 5 grms. of fat) of soaps from the determination of saponification value and of unsaponifiable matter is cooled, 5 ml. of potassium hydroxide solution (50° Bé.) added, and a solution of potassium permanganate (30 grms.) in water (650 ml.) added whilst the mixture is kept below 25°. After thorough shaking, the mixture is left overnight, and then decolorised with warm concentrated bisulphite solution and dilute sulphuric acid. The oxidation is said to yield a mixture of higher saturated acids, together with dihydroxystearic acid, sativic and linusic acids, azelaic, nonoic, propionic and other lower acids from oleic, linoleic, etc., acids. Extraction with petroleum spirit (b.pt. 40–60° C.) is stated to remove the higher saturated acids and nonoic acid from the hydroxystearic acids and dibasic acids formed in the oxidation. The acids recovered from the petroleum spirit are dissolved in water (200 ml.) containing a little ammonia, 10 per cent. ammonium chloride solution (30 ml.) is added, and the solution boiled with excess of magnesium sulphate solution, which precipitates the higher fatty acid salts and leaves magnesium nonoate in solution. The filtered magnesium salts and also the combined filtrates and washings are separately treated with dilute sulphuric acid and the acids obtained are submitted to a repetition of the magnesium salt precipitation. The second precipitates of magnesium salts thus obtained are reconverted into fatty acids, which are extracted with petroleum spirit, and recovered from the latter solution and dried until constant weight is obtained.

This method, which has received favourable notice in many quarters, depends for its success on a number of factors which are considered below. Bertram (*Chem. Weekblad*, **1927, 24**, 320) has admitted that it is not applicable to fats containing lauric or lower saturated acids or containing unsaturated acids (such as petroselinic, Δ^6-oleic acid), which yield lauric or similar acids when disruptively oxidised; thus, to this extent, like the Twitchell process, it is restricted in its range of application. For synthetic mixtures of palmitic, stearic, arachidic, oleic and linoleic acids Bertram obtained results agreeing to within 0·1–0·7 unit with the calculated percentages of saturated acids present.

Clearly, for complete success, the following conditions are essential:

(i) The oxidation of unsaturated acids must be complete;

(ii) Dihydroxy- or other polyhydroxy-stearic acids produced by the oxidation must be completely insoluble in the petroleum spirit employed, whilst higher fatty acids must be completely taken up by this solvent;

(iii) Lower fatty acids produced by the oxidation, and also dibasic acids (if not completely insoluble in the petroleum spirit) must be completely removed in the form of water-soluble magnesium salts.

In connection with the last point, we have found that magnesium nonoate and magnesium azelate are each sufficiently soluble to present no difficulty under the conditions given by Bertram for the magnesium salt separation, whilst the following data which we obtained with reference to magnesium laurate and myristate confirm

the statement of this author as to the limitations of the method as regards diminishing molecular weight of the higher saturated fatty acids:

Acid examined.		Fatty acid in filtrates from Bertram magnesium salt separation.	
	Grm.	Grm.	Percentage of acid taken.
Lauric	1·026	0·1425	13·9
Myristic	1·039	0·0190	1·8

The method is thus not suited for the determination of lauric acid, whilst myristic acid lies on the boundary line, and small proportions of this acid, when present with larger amounts of palmitic or stearic acid, or both, in a fat, can probably be dealt with fairly accurately in the magnesium salt separation, although losses of the order indicated must be expected to take place.

It seems to have been taken for granted hitherto that the oxidation of unsaturated acids by the Bertram process is complete, but we have found in all cases that the recovered "saturated" acids possess a small but definite iodine value. It is, therefore, desirable, as in the Twitchell method, to apply a correction based on this observed iodine value, with the result that, in many cases, the corrected figures obtained by the Bertram process are distinctly lower than either the uncorrected data or the values obtained by the fractionation method. Further, whilst it is logical to make this correction, the possibility must be borne in mind that in the case of the oxidation process it is possible for semi-oxidised products from oleic acid which do not show iodine absorption to be found in the final "saturated" acids; so that it is not certain that correction on the basis of observed iodine value will in this case allow for all the unsaturated acid derivatives which may be present in the final product.

The presence of semi-oxidised compounds of this type is probable, however, rather with permanganate-acetone oxidation (see below) than with the Bertram alkaline permanganate oxidation. In the latter case the first products of oxidation will be almost quantitatively dihydroxy- or polyhydroxy-stearic acids, and the important point here is the relative solubility of any of these acids and of the higher saturated fatty acids in the solvent employed for the final extraction of the latter.

We find that the petroleum spirit (b.pt. 40–60° C.) recommended by Bertram gives a satisfactory separation when no great amount of stearic or higher saturated acid is present, but, otherwise, it is difficult to ensure complete removal of stearic acid from the admixed dihydroxystearic acid by the use of this solvent, and in a number of experiments on beef tallow (*cf.* p. 356) we obtained low results. We studied the substitution of petroleum spirit (b.pt. 60–80° C.) and benzene for the lower-boiling petroleum spirit in this case, and found that, whilst the petroleum spirit of b.pt. 60–80° C. gave, on the whole, reasonably good results, benzene also dissolved some dihydroxy-stearic acid and gave a higher value for the saturated acids than the true one.

We came, consequently, to the conclusion that separation of higher saturated fatty acids from dihydroxystearic acid by selective solubility in an organic solvent is, perhaps, a weakness in the Bertram method, and we have endeavoured to use conditions of oxidation which will avoid, or reduce to a minimum, the formation of polyhydroxystearic acids. In the first place, we have carried out the oxidation with alkaline permanganate at a somewhat higher temperature (35–50° C.), when the dihydroxystearic salt initially produced is further broken down into suberic, octoic and oxalic acids (*cf.* Lapworth and Mottram, *J.C.S.*, 1925, **127**, 1987); there seems no object in restricting the temperature to below 25°, since the saturated fatty acid soaps are not attacked under the conditions of oxidation. By this means the amount of dihydroxystearic acid in the final product is considerably reduced. Alternatively, the fat itself, instead of the mixed fatty acid sodium salts, is oxidised in solution in acetone with anhydrous potassium permanganate, in which case polyhydroxystearic acids are not produced, the initial product of oxidation being an oxygenated complex which breaks down into azelaic and nonoic acids; the final products of oxidation of the fat are recovered and completely hydrolysed, and the higher saturated fatty acids are then separated by the magnesium salt procedure as given by Bertram. Typical experimental results obtained during the course of this study will now be given in illustration of the foregoing statements.

(i) Application of the Method as given by Bertram.—The specimens of beef tallow, olive oil and cod-liver oil used in testing the Twitchell lead salt alcohol separation were again employed, and, except for variation in the organic solvent, as described in Table IV, the conditions prescribed by Bertram (*cf.* p. 360) were carefully followed throughout:

Table IV.

Fat.	Solvent employed.	Saturated acids isolated. Gross. Per Cent.	Iodine value.	Corrected. Per Cent.	By fractionation analysis. Per Cent.
Beef tallow	Petroleum spirit, b.pt. 40–60° C.*	52·2	1·4	51·4	54·2
	,, ,, b.pt. 60–80° C.	53·5	3·3	51·5	,,
	,, ,, b.pt. ,,	56·6	3·2	54·6	,,
	Benzene	60·6	1·8	59·4	,,
Olive oil	Petroleum spirit, b.pt. 40–60° C.	13·3	3·8	12·7	12·6
	,, ,, ,, ,,	13·4	2·8	13·0	,,
Cod-liver oil	Petroleum spirit, b.pt. 40–60° C.	20·9	4·4	19·9	14·8
	,, ,, ,, ,,	18·3	5·8	16·1	,,

* Other results were obtained in which the gross yield of saturated acids was obviously too low.

As in the case of the Twitchell method, the correction for iodine value cannot properly be applied to the figures for cod-liver oil because of the complex mixture of unsaturated acids therein present; the results are too high, possibly owing to

the presence of saturated acids produced by oxidation from some of the characteristic unsaturated acids in the oil, and the method seems as little suited as the Twitchell procedure for marine animal oils.

(ii) BERTRAM'S METHOD, MODIFIED BY OXIDATION AT 35–50° C.—In the experiments illustrated in Table V, the directions and quantities given by Bertram (*loc. cit.*) were followed, except that (*a*) the alkaline permanganate was added to the soap solution at about 35–40° C., and the temperature allowed to develop up to, but not beyond, 50° C.; (*b*) the solvent used was petroleum spirit (b.pt. 60–80° C.); (*c*) unsaponifiable matter was not removed prior to oxidation of the soap solutions.

TABLE V.

Fat.	Saturated acids isolated.			By fractionation analysis. Per Cent.
	Gross. Per Cent.	Iodine value.	Corrected. Per Cent.	
Beef tallow	55·1	2·8	53·4	54·2
Cottonseed oil	27·2	4·5	25·8	25·0
,, ,, ...	28·7	8·8	25·9	
Borneo tallow	60·1	1·8	59·2	61·2
Cacao butter	59·4	2·0	58·1	58·8
Stillingia tallow	67·7	0·1	67·6	70·0*

* The fractionation analysis indicated the presence of about 1·5 per cent. of lauric and 3 per cent. of myristic acid.

(iii) OXIDATION OF AN ACETONE SOLUTION OF FAT WITH ANHYDROUS PERMANGANATE.—The fat (5 grms.) was weighed out accurately into a 500 ml. (or 1000 ml.) round-bottomed flask and dissolved in acetone (250 ml. or 500 ml., according to the degree of unsaturation of the fat). Potassium permanganate (20 grms.) was added in small portions, with frequent shaking and gentle boiling under a reflux condenser, followed by more vigorous boiling for half-an-hour, when it was set aside overnight; next day, more potassium permanganate (5 to 30 grms.) was similarly added, the mixture again refluxed for half-an-hour and the acetone then removed by distillation. The residue was decolorised and acidified, and the organic products were extracted by ether. The residue from the washed ethereal extract was completely saponified with alcoholic potassium hydroxide and the soaps reconverted into acids, which were then submitted to the magnesium salt separation as described in the Bertram method.

The amounts of acetone and permanganate used depended on the unsaturation of the original fat, but the following general proportions may be recommended for a quantity of 5 grms. of fat:—

Original fat (5 grms.).	Acetone.	Permanganate.	
		1st stage.	2nd stage.
Iodine value 40 or below (*e.g.* chocolate fats) ..	250 ml.	20 grms.	—
,, ,, 40–55 (*e.g.* tallows)	250 ml.	20 grms.	5–10 grms.
,, ,, 80–110 (*e.g.* olive oil, cottonseed oil)	500 ml.	20 grms.	15–30 grms.

The results obtained, calculated, as in the preceding cases, to the mixed fatty acids present in the fats oxidised, are given in Table VI.

TABLE VI.

Fat.	Saturated acids isolated. Gross. Per Cent.	Iodine value.	Corrected. Per Cent.	By fractionation analysis. Per Cent.
Beef tallow	{ 54·4 56·3	1·8 2·2	{ 53·3 54·9	54·2
Olive oil	13·9	3·7	13·3	12·6
Cottonseed oil	27·1	3·1	26·2	25·0
Borneo tallow	{ 62·7 66·3	3·5 6·7	{ 60·3 61·4	61·2
Cacao butter	59·3	1·4	58·4	58·8
Stillingia tallow	70·2	2·8	68·0	70·0*

* The fractionation analysis indicated the presence of about 1·5 per cent. of lauric and 3 per cent. of myristic acid.

Examination of Tables V and VI indicates that both methods give somewhat better figures than the Bertram method itself, although duplicate analyses still fail in some cases to give satisfactory agreement. With the Bertram oxidation carried out at 35–50° C., the experimental value for beef tallow is appreciably nearer to that determined by fractionation, and the other data in Table V, with the exception of those for Borneo tallow (the fatty acids of which contained 39 per cent. of stearic acid), are also within one unit of the percentage estimated by fractionation analysis.

The same order of agreement holds in Table VI (permanganate and acetone oxidation), and here the discrepancy in the case of Borneo tallow has disappeared; it is, however, noticeable that the more unsaturated oils now tend to give somewhat high figures for their saturated acid content, and this is probably to be attributed to the presence of traces of semi-oxidised unsaturated acids which cannot be corrected for by iodine value (*cf.* p. 362).

We have formed the following general conclusions regarding the oxidation processes for estimating saturated higher fatty acids:

(i) Although, in cases where palmitic and stearic acids are substantially the only saturated acids present, the (uncorrected) results obtained by following Bertram's method are usually close to the actual value, the saturated acids, as weighed, possess a small iodine value, and thus retain small proportions of unsaturated acids. When a correction is applied for the observed iodine value, the figures obtained for the saturated acids are frequently slightly low (1 to 2 units).

(ii) Alkaline oxidation of the soaps with permanganate at 35–50° C., or oxidation of the fats themselves in acetone with anhydrous permanganate, yields more consistent results than the low temperature alkaline oxidation proposed by Bertram.

(iii) When the saturated acids include none of lower molecular weight than palmitic (or myristic to the extent of not more than 5 per cent. of the total mixed fatty acids), the oxidation methods (ii) lead to results which should not be more than one unit lower than the true percentage, and which frequently lie well within

this limit. For such cases the modified methods suggested may be expected to yield somewhat more accurate results than the lead salt separation, even when the latter is carried out under the best conditions.

(iv) The oxidation methods are of no more use than the lead salt separations in the case of (*a*) fats containing notable amounts of lauric or lower acids (butters, nut oils, etc.) or (*b*) fats in which unsaturated acids other than oleic, linoleic and linolenic are present in quantity (*e.g.* marine animal oils, seed-fats of the *Cruciferae* or *Umbelliferae*).

It is obvious that, when oleic and linoleic acids only are known to be present, accurate estimation of saturated fatty acids permits the proportions of these unsaturated acids also to be deduced from the iodine value of the total mixed acids. In Table VII the data thus calculated from the results given in Table VI are compared with those obtained in the fractionation analyses.

TABLE VII.

Fat.	Iodine value.	Oxidation analysis.			Fractionation analysis.			
		Saturated acids.	Oleic acid.	Linoleic acid.	Saturated acids.	Oleic acid.	Linoleic acid.	Unsap. matter.
		Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Beef tallow ..	42·1	53·3	44·1	2·4	54·2	42·6	3·0	0·2
	,,	54·9	40·9	4·0	,,	,,	,,	,,
Olive oil	83·6	13·3	74·9	11·0	12·6	79·1	7·5	0·8
Cottonseed oil ..	104·7	26·2	24·8	48·3	25·0	29·3	44·9	0·8
Borneo tallow ..	32·3	60·3	38·8	—	61·2	37·9	—	0·9
	,,	61·4	37·7	—	,,	,,	—	,,
Cacao butter ..	37·1	58·4	39·6	1·8	58·8	39·0	2·0	0·2

Naturally, any error in the saturated acids figure frequently leads to magnified differences in the deduced values for oleic and linoleic acids [the case of olive oil is exceptional, since the 0·8 per cent. of unsaponifiable matter present in the fat has an iodine value of about 195; allowing for this, however, the values for oleic and linoleic acids become, respectively, 76·7 and 9·2 per cent., this discrepancy being the result of the difference between the respective observed percentages (13·3 and 12·6) of saturated acids].

If the oxidation method for determining saturated fatty acids could be relied upon to within one unit per cent., the values for oleic and linoleic acids could be calculated within about ± 2 units per cent. In conjunction with the iso-oleic acid determination, as proposed by Cocks, Harding and Christian (p. 368), a fairly rapid assay of the saturated acids, oleic and iso-oleic acids, and linoleic acid present in hydrogenated fats, would thus become available.

SUMMARY.—1. The Twitchell lead salt and alcohol separation and the Bertram oxidation method for the rapid estimation of saturated fatty acids have been tested on a number of fats which had also been analysed by the fractionation method.

2. Neither method is suitable for fats which contain lauric or lower saturated acids or solid unsaturated acids containing more than 18 carbon atoms in the molecule. When, however, the mixed fatty acids are composed of not more than 3–5 per cent. of myristic acid with palmitic, stearic, oleic (iso-oleic), linoleic and/or linolenic acids (this covers a very large section of natural fats, including tallows, palm oils and many liquid vegetable oils) both methods are useful within the following limits:

3. The lead salt separation, as originally given by Twitchell, gives results not more than 1 to 2 units per cent. below the actual content of saturated fatty acids, providing that (*a*) the proportion of lead acetate to saturated fatty acids is maintained as directed by Twitchell, (*b*) the temperature of the alcohol solution is not allowed to fall below 15–16° C. during cooling.

4. The oxidation method, as described by Bertram, leads to the production of dihydroxystearic acid in quantity, and of saturated acids which still possess a definite iodine absorption. Modifications in the oxidation are suggested which reduce or eliminate the dihydroxystearic acid formation, but the saturated acids finally obtained are still accompanied by small amounts of unsaturated acids. A correction should be applied for the latter, on the assumption that the iodine value observed is due to oleic acid.

5. The original Bertram procedure gives results within 1 unit per cent. of the calculated value in some cases, but in others the values obtained may be lower; the modified procedures yield more consistent and higher values in the instances which have been examined.

6. For the rapid determination of saturated fatty acids, we are inclined to prefer the modified oxidation methods to the lead salt separation, although parallel determinations by both procedures in their improved forms are probably safest.

7. If the saturated acids can be determined within one unit per cent., and oleic (iso-oleic) and linoleic acids only are present, the amounts of each of the latter can be estimated at once to within about ± 2 units per cent. from the iodine value of the original mixed fatty acids, iso-oleic acids being determined, when present (hardened fats), by the method suggested by Cocks, Harding and Christian.

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THE UNIVERSITY,
LIVERPOOL.

A New Method for the Determination of Solid Unsaturated Fatty Acids.

By L. V. COCKS, F.I.C., B. C. CHRISTIAN, Ph.D., A.I.C., and G. HARDING, F.I.C.

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The reliable determination of the solid saturated and solid unsaturated fatty acid contents of a fat or fat mixture is of great importance in a number of directions. Thus the knowledge of the proportions of these constituents is necessary in order to be able to follow the course of hydrogenation processes, and is of value in connection with (1) the consistence of margarine and pastry fats, and (2) the properties of soaps, as well as serving, in conjunction with other characteristics, as a means of recognising and approximately estimating partially hydrogenated oils in fat or fatty acid mixtures.

Until some six months ago, we, in common with very many chemists connected directly or indirectly with the oil and fat industries, employed the method given by Twitchell (*J. Ind. Eng. Chem.*, 1921, **13**, 806) for determining the solid unsaturated fatty acid content of hydrogenated fats. Admittedly, Twitchell, in his original publication, gave only one example of the determination of solid unsaturated fatty acids, namely, in hardened cottonseed oil. Following the publication of his method, however, we examined it from the point of view of determining the solid unsaturated fatty acid content of different partially hydrogenated fats, and then came to the conclusion that, even if it were not entirely satisfactory, it undoubtedly represented an improvement on the lead salt and ether method of Gusserow and Varrentrapp, which had been largely employed prior to the publication of Twitchell's lead salt and alcohol method.

Some eighteen months ago our attention was directed by our colleague, Dr. A. Taffel, in London, to the discordant results obtained by different workers when using the Twitchell method for the determination of the total solid fatty acid content of partially hydrogenated whale oil. After a preliminary examination of the cause of the disagreement between determinations made at different times and in different laboratories, Dr. Taffel concluded that the method could be improved by increasing the proportion of lead acetate used for the formation of the lead salts, see p. 372.

While our investigation was in progress, Grossfeld (*Chem. Umschau*, 1930, **37**, 3, 23; *Z. Unters. Lebens.*, 1930, **59**, 237–258) published a method which was based

on the use of more dilute alcohol than that employed by Twitchell for precipitating, washing and recrystallising the lead salts. Grossfeld showed that by his modification he could detect the addition to cocoa butter of small amounts of partially hydrogenated arachis oil, but, as the result of a series of experiments with varying proportions of solid unsaturated acids, he concluded that in no case did his method return more than 63 per cent., and in many cases not more than 40 per cent. of the total solid unsaturated acids present. Further, when tested on mixtures containing a large proportion of oleic acid, his method became less reliable for the detection of small proportions of partially hydrogenated fats, on account of the precipitation of the lead salts of the liquid acids at the same time as those of the solid acids.

We had made tests on the use of aqueous alcohol on somewhat similar lines to those described by Grossfeld, and concluded that, while a much larger proportion of the total solid unsaturated acids was obtained than by the Twitchell process, there was still definite under-estimation of these acids, and at the same time there was a distinct risk of separation of liquid acids with the solid acids. We decided, therefore, that we required a method that would estimate a greater proportion of the actual solid unsaturated acids present, and would, at the same time, be free from the somewhat uncertain precipitation of liquid acids in conjunction with the solid acids.

At the commencement of this investigation, it was realised, in view of the results obtained by Moore (*J. Soc. Chem. Ind.*, 1919, **38**, 320), Hilditch and Vidyarthi (*Proc. Roy. Soc., A*, 1929, **122**, 552), and other workers, that a variety of iso-acids would be formed in the ordinary hydrogenation process, in addition to the preponderating elaidic acid. As these other acids possess melting points which may range from that of oleic acid or lower, up to that of elaidic acid or higher, some definition should be given for purposes of differentiating between solid and liquid, and for this reason it was decided that acids which were still solid at 20° C. should be classified as "solid acids."

Experiments Employing Twitchell's Method.—In the first place tests on the fatty acids from naturally occurring oils by Twitchell's method showed that similar results to those published by him were obtained, duplicate tests being within $\pm 1{\cdot}0$ unit per cent. The application of his method, however, to fatty acids from partially hydrogenated whale and soya-bean oils gave much less consistent results, duplicates varying, in the case of the former oil, by ± 2 units per cent. for the saturated acids and ± 3 units per cent. for the solid unsaturated acids.

In order to trace the reason for this variability in the results, and to see whether even the maximum figures represented the total quantity of solid acids present, six entirely comparable determinations were started side by side, and the filtrate and washings from the first precipitation were collected and placed on one side, and the filtrate and washings from the second precipitation were likewise kept, but not mixed with those from the first precipitation. Each of the alcoholic solutions was concentrated in two stages, at the end of each of which the liquid

was allowed to stand overnight at 15–20° C., and then any solid material was removed by filtration. The characteristics of the acids separated in this way are summarised in the following table:

TABLE I.

	First Filtrate and Washings. Total amount of acids 37·9 per cent., expressed on original hydrogenated whale oil fatty acids.			Second Filtrate and Washings. Total amount of acids 14·5 per cent., expressed on original hydrogenated whale oil fatty acids.		
Fractions:	1A.	2A.	Residue A.	1B.	2B.	Residue B.
Character of separated fatty acids	Solid	Liquid	Liquid	Solid	Semi-solid	Liquid with some solid
Fatty acids expressed on original hydrogenated whale oil fatty acids, per cent.	6·25	2·1	29·05	6·2	4·3	4·0
Iodine value	77·3	88·8	96·3	62·7	71·7	80·8
Melting point °C.	23–25	—	—	30·0	20–25	—

Re-conversion to lead salts of the above solid acids from fraction 1A, and re-crystallisation once from a proportionately reduced quantity of 92–93 per cent. (by weight) alcohol gave 5·9 per cent. of solid acids (m.pt. 26·9° C.) and 0·35 per cent. of acids which were practically completely liquid at 20° C., both percentages being expressed on the total weight of hydrogenated whale oil fatty acids taken initially. Similar treatment of fraction 1B gave 5·0 per cent. of solid acids (m.pt., 32° C.) and 1·2 per cent. of acids which appeared to be almost entirely solid at 20° C.

It will be seen from the iodine values and melting points of the solid fractions, in conjunction with the results obtained by reconverting these fractions into lead salts and separating, that, while the solid acids are for the most part unsaturated, they evidently contain a small proportion of solid saturated acids, a fact which supports the findings of Hilditch and Priestman, see p. 360.

The above-described fractional separation not only demonstrates the large proportion of solid acids which are classified as "liquid" acids by the Twitchell process, but also serves to indicate in a roughly quantitative manner the minimum total amount of solid acids present in this sample of fatty acids from hydrogenated whale oil. Thus the total solid acids found by the Twitchell process, amounting on the average to 49·8 per cent., must be augmented by an amount of not less than 12·1 per cent., making a total solid fatty acid content of not less than 61·9 per cent. This last-mentioned figure may still be considered as a minimum, since, in the described method of separation, some solid acids will almost undoubtedly have remained dissolved as lead salts in the alcohol. This conclusion is confirmed by comparing the results obtained on this sample of whale oil when employing the new method to be described later (see p. 376).

Similar evidence of under-estimation by the Twitchell method was obtained by examining the filtrate and washings from the recrystallisation of the lead salts

of a series of Twitchell determinations on the fatty acids from partially hydrogenated soya-bean oil. From an initial separation of 5·3 per cent. solid acids, 3·5 per cent. solid acids (m.pt. 35° C.; iodine value, 80·0) were obtained on repeating the process. It was, therefore, clear that distinct amounts of solid unsaturated acids were escaping estimation by Twitchell's method.

In order to obtain further evidence regarding the quantitative character of this under-estimation of the solid unsaturated acids, the Twitchell method was applied to artificial mixtures of known composition. Representative solid unsaturated acids were prepared by partially hydrogenating the liquid acids from cottonseed oil so that practically no saturated acids were formed. The solid unsaturated acids (m.pt., 39° C.; iodine value, 83·6) which were separated from the remaining liquid acids by precipitation and two recrystallisations as lead salts from alcohol were mixed with varying proportions of cottonseed fatty acids, and tested by Twitchell's method with the following results:

TABLE II.

ARTIFICIAL MIXTURES OF COTTONSEED OIL FATTY ACIDS AND SOLID UNSATURATED ACIDS.

	Solid unsaturated acids, per cent.					
Added	Nil	5·0	10·0	10·0	15·0	20·0
Found by Twitchell method	0·1	0·9	0·5	3·9	6·3	8·8

The solid unsaturated acids were thus very materially under-estimated, in some cases as much as 10 per cent. being present almost without detection, and in all cases the return being well under 50 per cent.

The under-estimation of this large proportion of the solid unsaturated acids by the Twitchell process was evidently due to the solubility of the lead salts in the alcohol. The influence of this factor, so far as the different lead salts are concerned, can be seen from the following table:

TABLE III.

SOLUBILITIES OF THE LEAD SALTS OF FATTY ACIDS IN 92–95 PER CENT. (BY WEIGHT) ALCOHOL EXPRESSED IN TERMS OF WEIGHT OF FATTY ACIDS IN 100 C.C. SOLUTION.

Fatty acid or fatty acid mixture.		Approx. solubility in grms. per 100 c.c.	Temp. °C.
Myristic		0·0175*	12
Palmitic		0·010*	16·5
Mixture of 83 per cent. myristic and 17 per cent. palmitic		0·03	17·5
Mixture of 60 per cent. palmitic and 40 per cent. solid unsaturated C_{18} acid obtained from hydrogenated cottonseed oil by the Twitchell process—			
Palmitic constituent	0·02		17·5
Solid unsaturated constituent	0·06		
Total..	——	0·08	
Elaidic		0·214†	20–22
Mixed iso-oleic acids from cottonseed oil		0·454	17·5
Mixed solid acids from hydrogenated whale oil fatty acids (iodine value 57·5) by the Twitchell process		0·20	17·5

* Figures given by Twitchell, *loc. cit.* † Figure given by Grossfeld, *loc. cit.*

The above figures demonstrate the relative solubility of the different lead salts in alcohol and serve as an indication of the large losses of solid unsaturated acids which can take place.

Examination of Means for Improving the Accuracy of the Determination of the Solid Unsaturated Acids.

With the object of finding a means of reducing the loss of solid acids which occurs in the Twitchell determination, the influence of the following factors, in addition to the effect of aqueous alcohol already referred to on p. 369, was examined: (1) The amount of lead acetate for precipitation; (2) The use of lead acetate solution for washing and recrystallisation; (3) Conditions of cooling; (4) Single crystallisation of lead salts; (5) Use of solvents other than ethyl alcohol.

1. The Effect of the Amount of Lead Acetate employed in the Precipitation.—As already mentioned on p. 368, an improved yield of solid acids could be obtained in the case of partially hydrogenated whale oil by increasing the amount of lead acetate used for the precipitation. The extent of the improvement, however, even when the amount of lead acetate used corresponded to 140 per cent. of that required theoretically by all the fatty acids present, was only small, and amounted to an additional 3 to 4 per cent. of solid acids. Generally speaking, the use of this equivalent of lead acetate was highly desirable for mixtures containing a preponderance of solid acids, but with those containing less than 25 per cent. of solid acids it was most important to use about 100 per cent. excess of lead acetate above that required for the solid acids only, since, if this amount were exceeded, lead salts of the liquid acids were precipitated in quantity.

(2) The Effect of the Use of Lead Acetate Solution for Washing and Recrystallisation.—It was found experimentally that a concentration of 1·5 per cent. to 2·0 per cent. of lead acetate, $Pb(CH_3.COO)_2.3H_2O$, represented the optimum concentration for depressing the solubility of the more soluble lead salts of the solid fatty acids. The results obtained, however, even when a quantity equivalent to 140 per cent. of that theoretically required by the total fatty acids was used initially in the precipitation, were only slightly more satisfactory in respect of the return of solid acids, and still showed that very appreciable losses of these acids occurred.

(3) Conditions of Cooling.—By allowing the crystallisations of the lead salts in the first and second precipitations to continue for varying lengths of time it was shown that gradual precipitation took place, but even after five days at 15–20° C., the yields obtained were still distinctly low.

Variations in the temperature of cooling showed that it was unsafe, at any rate with certain mixed fatty acids, to cool to 0° C. on account of the precipitation of the lead salts of liquid acids. Generally speaking, it appeared that a cooling temperature of 15–20° C., which should be reached gradually, was the most satisfactory, and as this coincided with Twitchell's conditions, it was concluded that no improvement in the process could be effected by changing the temperature of cooling.

(4) SINGLE CRYSTALLISATION OF LEAD SALTS.—The possibility of being able to estimate the solid fatty acids without having to recrystallise the lead salts was investigated fully, in spite of the fact that Twitchell (*loc. cit.*) had indicated in his original paper that no amount of washing without recrystallisation would render the lead salts of the solid fatty acids sufficiently free from liquid acids. It was found that by single crystallisation followed by very thorough washing with alcohol, the amount of retained liquid acids could in many cases be reduced to a reasonable limit, but in others this seemed impossible. However, the volume of alcohol necessary was so large as to cause distinct loss of the lead salts of the solid unsaturated acids. This loss of solid unsaturated acids by washing, coupled with the necessity for such thorough washing, prompted the search for a more suitable solvent than alcohol.

(5) THE USE OF SOLVENTS OTHER THAN ALCOHOL.—The possibility of using solvents other than alcohol for effecting the separation between solid and liquid acids has been considered and applied by various workers in the past, but, for one reason or another, the processes have not proved entirely satisfactory. Attempts were, therefore, made to see if some improved method could be devised.

In the first place, experiments on the solubility of the lead salts prepared from partially hydrogenated whale oil fatty acids by three crystallisations from alcohol, showed the following results:

TABLE IV.

Solvent.	Fatty acids in solution per 100 c.c. at 15–17° C. Grm.
Ethyl alcohol (92–95 per cent. by weight) ..	0·035
Methylated ether	0·007
Petroleum spirit, b.pt. 40–60° C.	0·005
Acetone	0·014

As petroleum spirit seemed to be the solvent which dissolved the lead salts of the solid acids to the least extent, and as it readily dissolved the lead salts of the liquid acids, a further series of comparisons on the solubility of the lead salts of the solid acids from hardened whale oil in 92–95 per cent. (by weight) alcohol and in petroleum spirit was carried out on the lead salts which had only been precipitated once and washed with the minimum quantity of 92–95 per cent. (by weight) alcohol. The results of successive extraction treatments by the two solvents on such lead salts are shown in the following table:

TABLE V.

	Approximate solubility of lead salts expressed in grms. of fatty acids in solution per 100 c.c. of solvent at 15–17° C.	
	Alcohol (92–95 per cent. by weight).	Petroleum spirit (b.pt. 40–60° C.)
First extraction	0·560	0·180
Second ,, ...	0·210	0·021
Third ,, ...	0·137	0·022
Fourth ,, ...	0·094	0·012
Fifth ,, ...	0·074	0·010

The above figures, apart from the first extraction, in which probably both solvents were removing a certain proportion of retained liquid acids, show that the solubility of the lead salts is of the order of seven to ten times greater in the 92–95 per cent. (by weight) alcohol than in the petroleum spirit.

The possibility of using petroleum spirit as the sole solvent for the determination was considered, but it soon became clear that some other solvent must be used, at any rate, for the formation of the lead salts. Methylated ether, on account of the fact that its power for dissolving the lead salts of the solid acids is only slightly greater than that of petroleum spirit, was also tested without the use of any other solvent for the formation of the lead salts, but the results did not prove satisfactory. The use of petroleum spirit was, therefore, tried, after forming the lead salts in alcoholic solution, as in this way the lead salts could be obtained in a readily-washable, crystalline condition, in contrast with their sticky and unmanageable character when prepared by double decomposition from aqueous solution (*cf.* Gusserow–Varrentrapp). This formation of the lead salts in alcoholic solution, and their subsequent treatment with petroleum spirit, forms the basis of the new method of determining the solid acid content of mixtures, and the experimental evidence upon which the new method was devised will now be described.

Basis of the New Method of Determination.—The advantages to be anticipated from a method involving the formation of the lead salts in alcohol, followed by thorough washing with petroleum spirit, were that, by controlling the quantity of alcohol used for the precipitation in relation to the amount of acids employed in the test, the loss of solid unsaturated acids through the solubility of their lead salts in alcohol should be reduced to a minimum, and washing of the lead salts with petroleum spirit should remove the liquid acids and their lead salts without dissolving out more than mere traces of the lead salts of the solid saturated and unsaturated acids. Experiments showed, however, that the alcohol used for the precipitation of the lead salts must not contain too great a concentration of liquid acids, otherwise the lead salts of these acids were precipitated in large quantities and became difficult to remove even with petroleum spirit. Furthermore, there was evidence that when the co-precipitation from alcohol of the lead salts of the solid and liquid acids took place, appreciable quantities of mixed lead salts of solid and liquid acids were formed, with the result that the efficiency of the petroleum spirit washing treatment was considerably impaired.

Preliminary experiments indicated that on naturally occurring unhardened fats, the new procedure involving the precipitation of the lead salts from alcoholic solution and washing the precipitate with petroleum spirit gave an iodine value of the separated fatty acids somewhat higher than that generally found by Twitchell's method. Thus, with the latter, the apparent amounts of solid unsaturated acids ranged from 0·1 to 0·3 in natural oils and fats (excluding tallow), whilst with the new method the amounts ranged from 1·9 to 3·4. On the other hand, however, with samples of partially hydrogenated whale, olive, cotton and

soya-bean oils, the amounts of solid unsaturated acids were from 1·5 to three times greater by the new method than by Twitchell's method.

In order to test the value of the new procedure in respect of its efficiency for estimating solid unsaturated acids, determinations were carried out on artificial mixtures containing known amounts of these acids. While the results were much closer to the actual than by the Twitchell method, there still appeared to be an appreciable under-estimation of the solid unsaturated acids. Stagewise concentration of the alcoholic filtrate, followed by overnight cooling periods at 15–20° C., did not give any further deposit of the solid acids, but examination of the material removed by the petroleum spirit washing treatment showed that a small quantity of solid acids had been dissolved. These solid acids could be recovered by removing the petroleum spirit and dissolving the residue in a small proportion of boiling alcohol containing 0·5 per cent. acetic acid, from which solution the lead salts of the solid acids precipitated almost immediately on cooling. Typical examples of the amounts, melting points and iodine value of the solid acids which were recovered in this way from tests which had been carried out by the new procedure involving washing with petroleum spirit, are given in the following table:

TABLE VI.

From:	Fatty acids. Iodine value.	Solid acids dissolved from lead salts by petroleum spirit.		
		Expressed on total fatty acids.	M.pt. °C.	Iodine value.
		Per Cent.		
Partially hydrogenated whale oil ..	57·5	6·0	18–20	89·0
,, ,, cottonseed oil ..	74·7	7·4	29–30	81·0
,, ,, olive oil ..	35·7	3·0	27	73·9
,, ,, soya-bean oil ..	86·6	7·2	25	84·0

The cause for this removal of solid fatty acids by the petroleum spirit washing appears to be either the presence of certain particular solid unsaturated acids or the formation of mixed lead salts. The latter seems to be the more probable explanation, as the amount of solid acids which pass into solution in the petroleum spirit varies not only with the particular sample of partially hydrogenated fat, but also with the proportion of fatty acids and alcohol in the first precipitation. It, therefore, became necessary to include this additional procedure in the method, so as to be sure that appreciable proportions of solid acids were not escaping estimation.

A feature of great importance in this new method is the avoidance, as far as possible, of the formation of insoluble lead oleate, and, therefore, the proportions of fatty acids, lead acetate and alcohol must be rigidly adhered to, and care must be taken to prevent the temperature falling below 15° C. during the precipitation of the lead salts from alcohol.

DESCRIPTION OF THE NEW METHOD OF SOLID FATTY ACID DETERMINATION.—In all cases a quantity of 3·5 grms. of the freshly prepared fatty acids* is dissolved in 50 c.c. of 92–93 per cent. (by weight) alcohol and (except with liquid oils or mixtures containing less than 25 per cent. of solid acids), 3·45 grms. of lead acetate, $Pb(CH_3COO)_2.3H_2O$, is dissolved in 50 c.c. of similar alcohol. (With mixtures containing less than 25 per cent. of solid acids the same weight of freshly-prepared fatty acids is taken, but only 1 grm. of the lead acetate is dissolved in the alcohol.) Both solutions are heated to boiling point, and that containing the lead acetate is poured into the one containing the fatty acids. After mixing and again heating to boiling, the solution is allowed to cool slowly and evenly and to stand overnight† at 15° C. to 20° C., which limiting temperatures must be adhered to strictly (being checked by a maximum-minimum thermometer).

The next morning the mixture is stirred and filtered from the alcohol as completely as possible on a 10 cm. Buchner funnel, which is then transferred and fixed to a clean filter flask. The lead salts on the Buchner funnel, and any remaining in the precipitating vessel, are washed with 100 c.c. of petroleum spirit (b.pt. 40–60° C.), using amounts of 20 c.c. at a time, and the washings are then distilled from a water-bath to remove the petroleum spirit completely. The residue is dissolved by boiling under a reflux condenser with 20‡ c.c. of 92–93 per cent. (by wt.) alcohol containing 1 drop (0·05 grm.) of glacial acetic acid, added from a pipette. The solution is allowed to crystallise at 15–20° C. for 3 hours. The lead salts of the solid acids which precipitate readily under these conditions from the alcohol are filtered and washed with 20 c.c. of cold 92–93 per cent. alcohol, and the solids remaining behind on the filter paper are worked up with the main bulk of the lead salts of the solid acids left in the Buchner funnel after washing with petroleum spirit, the method being to decompose the lead salts with dilute nitric acid in the presence of methylated ether, washing the latter free from mineral acid and removing the solvent.

The total solid fatty acids are weighed and the whole, or a portion, is used for iodine value determination, from which the amounts of solid saturated and solid unsaturated acids can be calculated on the basis that the latter will have a mean iodine value of 90, unless evidence is available to show that some other iodine value should be adopted, *e.g.* erucic acid from rape oil, iodine value 75·5.

* Consideration has been given to the possibility of starting the estimation from glycerides. It has been shown that neutralisation of the alkali used for alcoholic saponification cannot be effected with acetic acid without giving rise to low results. Sulphuric and oxalic acids, on account of the relative insolubility of their sodium salts in alcohol, are much superior for the purpose, but, as yet, it has not been established that the results obtained are so reliable as those in which the freshly prepared fatty acids are employed.

† In the event of a result being required as quickly as possible, the cooling period may be reduced to 2½ hours, half-an-hour being taken for the mixture to cool in air on an asbestos mat, and two hours subsequent cooling in water at 15–20° C. If this procedure is adopted the yield of solid acids will generally be some 2 per cent. low in comparison with the method of cooling overnight.

‡ In exceptional cases of very slightly hardened fats containing high proportions of liquid acids the lead salts may separate in a liquid or semi-liquid condition when only 20 c.c. of alcohol are used. In such instances the quantity of alcohol, containing the same proportion of acetic acid, must be increased until no separation of the lead salts occurs in the liquid condition.

COMPARISON BETWEEN THE NEW METHOD AND TWITCHELL'S METHOD. The figures in the following table represent a summary of the comparisons that have been carried out between the new method and Twitchell's method for the determination of the solid saturated and solid unsaturated fatty acid contents in naturally occurring and partially hydrogenated oils and fats.

TABLE VII.

Fatty acids from naturally occurring oils.	Iodine value of fatty acids.	Solid saturated acids.		Solid unsaturated acids.	
		Twitchell's method.	New method.	Twitchell's method.	New method.
		Per Cent.	Per Cent.	Per Cent.	Per Cent.
Olive	85·6	11·0*	12·3	0·3	1·9
Cottonseed	107·0	23·6	24·2	0·1	1·9
Soya-bean	138·5	11·7	11·9	0·1	2·1
Palm	53·7	46·9	47·4	0·3	3·4
Tallow	46·5	48·7	48·8	1·7	5·6
Coconut	9·1	47·5	63·4	nil	1·3
Rape	105·8	4·4	1·2	47·5	52·2
Fatty acids from partially hydrogenated oils.					
Whale	57·5	37·7	40·0	12·1	27·0
Whale	72·4	26·6	28·7	18·0	33·5
Whale	37·2	52·1	57·4	9·8	16·8
Cottonseed	74·7	26·6	29·6	15·2	34·4
Cottonseed	74·3	30·0	30·1	15·3	25·6
Cottonseed	80·1	26·5	26·9	23·1	32·3
Olive	80·0	15·1	14·9	5·9	20·8
Olive	73·5	21·5	21·8	12·6	22·6
Olive	35·7	62·3	61·2	11·5	26·2
Soya-bean	86·6	17·6	18·4	33·1	38·6
Soya-bean	59·6	34·3	34·4	32·0	39·5
Arachis	78·4	16·5	19·9	26·9	53·0
Arachis	62·4	29·7	32·2	30·3	45·4

* Using half the concentration recommended by Twitchell.

The above series of comparisons shows that, as a means of determining solid saturated acids in naturally occurring oils, containing no acids of lower molecular weight than myristic, the new method is as satisfactory as Twitchell's method. From the coconut oil result it will be seen that the method returns more lauric acid than the Twitchell method, but even so, its estimation of this acid is not complete. The most important feature, however, of the new method is the way in which it enables the solid unsaturated acids to be estimated to a much greater degree of accuracy than is possible by Twitchell's method.

From the evidence submitted it will be realised that the new method will, so far as solid unsaturated acids are concerned, involve two small balancing errors, the first depending on the solubility of the lead salts of the solid unsaturated acids in the alcohol used for the precipitation, and the second on the fact that the solid saturated acids from naturally occurring fats appear to be contaminated with a small amount of liquid acids (unless such amounts of solid unsaturated acids are naturally present). The significance of the former loss is not great in mixtures

containing appreciable proportions of liquid acids, as these appear to exert a marked depressing influence on the solubility of the lead salts of the solid unsaturated acids in the alcohol. The small amount of the loss in this direction is shown by the difficulty in obtaining any solid acids by concentrating and cooling the alcoholic liquor after removal of the insoluble lead salts. In practice, in all but extreme cases with either very small amounts of liquid unsaturated or very small amounts of solid unsaturated acids, the two errors tend to balance one another.

In order to obtain information regarding the degree of accuracy that may be expected from the new method, firstly, artificial mixtures of cottonseed oil fatty acids and solid unsaturated fatty acids isolated in the manner referred to previously (p. 371); and, secondly, artificial mixtures of palm oil fatty acids and solid unsaturated acids prepared from the liquid acids of soya-bean oil by partial hydrogenation and then separation under conditions similar to those used in the new method of determining solid unsaturated acids, were tested with the following results:

TABLE VIII.

	Artificial mixtures made to the following composition.		Composition found by new method.	
	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.
Cottonseed fatty acids ..	—	—	21·2	3·3
Mixtures	22·6	5·0	22·3	8·7
	21·3	12·1	21·6	15·1
	19·4	23·2	20·1	21·7
	16·4	40·0	17·1	36·8

For comparison with the Twitchell method see Table II. on p. 371.

TABLE IX.

	Artificial mixtures made to the following composition.		Composition found experimentally.			
			Twitchell's method.		New method.	
	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.
Palm-oil fatty acids	—	—	44·9	0·3	45·3	3·8
Solid unsaturated acids (iodine value 82) from soya-bean oil ..	—	—	7·0	61·9	6·1	70·8
Mixtures	44·7	2·0	44·2	0·7	45·1	5·6
	43·6	5·0	42·4	1·4	43·6	6·5
	38·2	20·0	37·0	7·6	36·3	17·4

From each of the series of tests it will be seen that with the smallest additions of solid unsaturated acids there is an apparent over-estimation, due to the fact that

the original mixtures to which no solid unsaturated acids had been added show some 3·5 per cent. of apparent solid unsaturated acids. These results represent extreme cases in one direction. In the other direction the extreme case is covered by the tests on the solid unsaturated acids from the soya-bean oil. The fact that the new method returns only 9 per cent. more solid unsaturated acid than does the Twitchell method, and shows an under-estimation of some 23 per cent., is due largely to the absence of liquid acids, which normally depress to a marked degree the solubility of the lead salts of the solid unsaturated acids in alcohol. From data collected on these solid unsaturated acids, prepared from the liquid acids of soya-bean oil by partial hydrogenation, it is considered that some 3–4 per cent. consisted of oxidised acids and some 3–4 per cent. of liquid unsaturated acids, so that the loss of solid unsaturated acids by the new method is probably about 16 per cent., in comparison with 25 per cent. by the Twitchell method. The accuracy of the new method is such that, for determining intermediate amounts of solid unsaturated acids of the order usually occurring in partially hydrogenated fats, either alone, or as an appreciable proportion of fat mixtures, it is considered to give results which are at least within 3·0 units per cent. of the actual solid unsaturated acid content (m.pt. 20° C. or above). In contrast with this, the Twitchell method cannot be relied on to anything like the same degree of accuracy, as the under-estimation is generally of the order of 10 units per cent., and in certain instances may be much greater.

The new method, when applied to rosin alone, showed a return of 56–58 per cent. of "solid" acids. In admixture with fatty acids, however, this figure was not proportionately maintained, a fact which may be connected with the unsaponifiable matter. In general, therefore, the degree of accuracy of the method is impaired somewhat by rosin, the presence and amount of which can readily be determined.

THE SEPARATION OF SOLID FROM LIQUID ACIDS BY MEANS OF SALTS OTHER THAN THOSE OF LEAD.—During the present investigation there have been several indications that lead salts were not ideal for the separation of solid and liquid acids by means of a fractional precipitation or crystallisation method, on account of the possibility of the formation of mixed lead salts containing both solid and liquid acids. Although it has been shown that, by adhering to definite concentrations of acids and precipitating salt, this mixed salt formation can be reduced to a minimum, it was thought that some univalent metal salt might be preferable. Thus, methods based on the use of silver and thallium salts for effecting the separation were subjected to a preliminary examination, but the results obtained did not seem to warrant their use in preference to the lead salts.

SUMMARY.—(1) The marked extent to which Twitchell's method under-estimates the solid unsaturated acids in partially hydrogenated fats has been shown, firstly, by the separation of solid acids which escape determination by that method; and secondly, by carrying out tests on artificial mixtures of known composition.

(2) The marked degree of under-estimation of the solid unsaturated acids by the Twitchell method is almost entirely due to the solubility of the lead salts of these acids in the 95 per cent. (by weight) alcohol employed for precipitation, crystallisation and washing.

(3) Certain modifications in Twitchell's method have been found to improve the accuracy to only a small extent. However, by means of the new method, involving the use of petroleum spirit (b.pt., 40–60° C.), in conjunction with the formation of the lead salts in alcohol, as described on pp. 374 and 376, a much more accurate determination is possible in a somewhat shorter time.

(4) Thus: (*a*) in mixtures containing solid unsaturated acids Twitchell's method only slightly under-estimates the solid saturated acids (*cf.* Hilditch and Priestman (*loc. cit.*) for naturally occurring fats), and materially under-estimates the solid unsaturated acids (Table I, p. 370, Table II, p. 371, Table IX, p. 378), generally to the extent of some 10 units per cent., and sometimes even more; (*b*) the new method, however, gives a rather more accurate return of solid saturated acids, and a definitely more accurate estimation of the solid unsaturated acids, which are determined, in all but extreme cases not likely to be met with in practice, to within 3 units per cent. of the actual.

In conclusion, we wish to thank Messrs. Lever Bros. Ltd. for permission to publish the results of this investigation.

RESEARCH LABORATORIES,
PORT SUNLIGHT.

The Rising of Fat in Milk.

BY GUNNER JØRGENSEN.

As a supplement to the experiments of Stock (ANALYST, 1930, **55**, 535), I should like to give the results of some tests, just completed, which were made in order to answer a question, raised by the police, as to the probable fat content of a sample drawn from the bottom tap of a churn containing milk which had been standing for about one hour.

From a general consideration of the question it appears that the depth of the layer of the very poor milk formed after a certain time at the bottom does not depend particularly on the depth of milk in the churn; so that, whether this is, for instance, 20 or 100 cm., the rise of fat in the lowest mm. or cm. of the milk will take place at virtually the same rate for the same milk under similar conditions. On the other hand, the distance of the bottom tap from the base of the churn has a bearing upon the quantity of fat in a small quantity of the milk drawn, as has also the velocity with which the milk is drawn. By slow drawing, the milk from the layer in front of the tap will be able to run out without getting mixed with the surrounding

layers; but, if the withdrawal takes place quickly, some milk from the surrounding layers, particularly the upper ones, will be drawn down to the tap and cause too high a fat-content in the sample. Furthermore, the proportions between the diameter of the churn and the quantity of milk drawn, will—so long as only the very lowest layers of milk have given up a considerable part of their fat—have an influence upon the amount of fat in the sample drawn.

In conformity with these considerations, I have made the tests by using two glass-vessels of different size and form. In the narrower of these vessels 3 decilitres of milk reached a height of 35 cm., whilst 15 decilitres of milk in the wider vessel reached a height of 23 cm.

The sides of the lower parts of these vessels were vertical, and were fitted with glass tubes with rubber tubing and pinch-cocks. After the milk had stood for a recorded time, 15 ml. were drawn off and tested. This volume represented, for the narrow glass, a height of about 3 cm.; and, for the wider glass, about 0·8 cm., measured from the top of the glass-tube, the volume of which was only 1·5 ml. For this reason the quantity of fat separated from this volume of milk has very little influence upon the fat content of the milk above.

The milk used, containing 3·75 per cent. of fat, had been heated at 50° C. for a short time and then re-cooled.

The samples drawn contained:

After standing for	The narrow glass. Per Cent.	The wide glass. Per Cent.
1 hour	2·90	1·35
2 ,,	2·60	1·10
24 ,,	0·25	0·15

From the upper layers in the vessel with 15 decilitres of milk, samples of different amounts were drawn through the glass tube after standing for 24 hours.

The distances of the samples from the bottom of the vessel were:

Cm.	Fat. Per Cent.
15·0–15·4	1·45
6·0–15·0	1·45
2·0– 6·0	0·95
0·8– 2·0	0·35
0 - 0·8	0·15

A comparison of these results with Tables VI–IX of Stock shows, that, after one hour, Stock found a considerably lower decrease of fat in the samples taken from the bottom tap than after two hours, whilst I find the greatest decrease after the first hour. There is no doubt that this discrepancy would have disappeared if Stock had placed the tap in the middle of the bottom of the churn and not one-and-three-quarter inches (=4·5 cm.) above the bottom. Thereby he would undoubtedly have found a greater decrease for the contents of fat in the lower part of the milk than he did.

Stock's tables also show a very considerable difference for the velocity with which the fat rises in his four samples of milk.

The following table gives the percentage alterations in contents of fat:

	Stock's test.				Jørgensen's tests.		
	During 1 hour.		During 2 hours.			Decrease.	
	Decrease, bottom-tap.	Increase, top-milk.	Decrease, bottom-tap.	Increase, top-milk.	Glass-vessel.	During 1 hour.	During 2 hours.
VIII	nil	0·05	0·05	0·35			
VII	0·02	0·15	0·12	0·57	Narrow	0·85	1·15
VI	0·22		0·97	2·15	Wide	2·40	2·65
IX	0·21	1·33	0·77	5·23			

Finally, a few words about the alteration in the contents of the solids-not-fat mentioned by Stock. In the fat-free "top milk" in his Table IX the contents of solids are calculated as follows:

	Fat in the corresponding milk.	
	Per Cent.	Per Cent.
Immediately	9·39	3·32
After standing for 20 minutes	9·28	3·42
40 ,, ...	9·31	3·62
1 hour	9·36	4·65
1 ,, 20 minutes ..	9·42	5·85
1 ,, 40 ,, ..	9·41	7·57
2 hours	9·68	8·55
2 ,, 20 minutes ..	9·67	10·05
2 ,, 40 ,, ..	9·60	10·95

According to this there is no visible increase of solids-not-fat for an augmentation of fat from 3·22 to 7·57 per cent., but for the samples with higher percentages of fat the increase suddenly becomes fairly constant.

On studying the figures in Tables VI–IX, recorded immediately after the placing of the milk in the churn, although these figures for each table should be the same, yet one observes a difference between the percentages of fat, up to 0·10 (Table IX), and between the total solids, up to 0·27 (Table VII). We may then take it for granted, that there may be differences in the figures of solids-not-fat up to 0·37 per cent., so that, in my opinion, there is, in the results given by Stock, nothing to show that an accumulation of fat in the upper layers of milk does involve an increase of solids-not-fat.

I do not, however, regard it as out of the question for such an alteration to take place, because the globules of fat are believed to be inclosed in a thin film of albumin. On the other hand, the milk fat will be oxidised by drying in the air, and, therefore, an increase of weight will take place, but this increase may be considered of about the same magnitude for the fat-containing solids and for the fat, and, consequently, the weight of solids-not-fat should be about the same for a rich and for a poor milk.

V. Stein's Laboratory, Copenhagen.

The Examination of Eggs suspected of being "Preserved."

By JOHN RALPH NICHOLLS, B.Sc., F.I.C.

Section 3 of the Agricultural Produce (Grading and Marking) Act, 1928, makes it unlawful "to sell or expose for sale any egg which has been subjected to any process of preservation unless the egg is marked in the prescribed manner." The Minister of Agriculture and Fisheries has power to "exempt from the operation of this section eggs preserved by any process with respect to which he is satisfied that the marking of eggs preserved by that process cannot be enforced." Difficulty of enforcement occurs where it is not possible to ascertain by examination whether eggs have, in fact, been preserved by any process; and the Minister of Agriculture issued an Order, dated 16th October, 1928, exempting eggs preserved by "cold storage and by chemical storage." The latter term is used in a limited sense, and is defined in the Act as "storage for the purpose of preserving eggs by any process which does not alter the composition of the shells, including storage in any gas, vapour or gaseous mixture."

The effect of this Order is to limit the present operation of Section 3 to eggs preserved by processes which alter the composition of the shell, and, in accordance with the Agricultural Produce (Grading and Marking) (Eggs) Regulations, 1928, Statutory Rules and Orders No. 984, such eggs must be marked "Preserved."

For the examination of samples taken under this Section it is necessary to have means for detecting when the shell's composition has been altered. The present paper gives the results of tests carried out with this object in view.

Methods of Preservation.—The methods commonly in use, other than those included in the above-mentioned exemption, may be divided into three classes:—(1) Treatment with oil or similar fluid. (2) Immersion in sodium silicate solution. (3) Immersion in lime water.

Eggs preserved by these processes will be referred to as oiled, silicated, and limed eggs, respectively.

(1) *Preservation with Oil.*—In this process the eggs are immersed in oil or other similar fluid, so that on removal each egg is covered with a thin film. Among the substances which are stated to be suitable for this purpose are the following:—Mineral oil (with or without waxes, gums and resins), molten paraffin wax, poppy oil, linseed oil, tung oil, drying oil with or without resins and thinners. Patents have been granted for such protective coatings as camphor, celluloid and amyl acetate after a first coating of gelatin, collodion after a first fatty coating, and synthetic resins, but no eggs covered with such protective coatings have been encountered.

In some cases the eggs are dipped in the cold liquid; in others, hot oils at 210–260° F. are used, the eggs being immersed for not more than a few seconds. One preserving medium for home use is sold as a grease which is to be placed in the palm of the hand and in which the egg is rolled until completely covered.

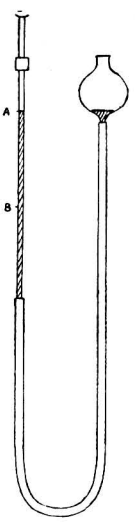
In connection with this process of preservation it is to be noted that eggs intended for cold storage are sometimes "processed" or "sterilised" by dipping them in hot oil, in order to prevent evaporation of water from the contents of the eggs during the time they are in cold store. In such cases the process is not primarily designed to replace cold storage, but rather to prevent deterioration in the quality of the eggs. Whether such eggs are entitled to be sold without being marked "PRESERVED" is doubtful.

(2) *Preservation in Sodium Silicate Solution.*—The eggs are completely immersed in a 3 to 10 per cent. solution of sodium silicate (water-glass), and are usually allowed to remain therein until required. On removal, the eggs are generally rinsed before sale.

(3) *Preservation in Lime Water.*—This is similar to (2), the solution of sodium silicate being replaced by saturated lime water. An "ice" of calcium carbonate forms on the surface of the bath and retards the diminution of strength of the alkaline liquid by the air.

MECHANISM OF PRESERVATION.—A freshly-laid egg is warm and the contents completely fill the shell. On cooling, slight contraction occurs and an air space forms between the egg membrane and shell membrane. This air has been sucked through the pores of the shell. When exposed to the air the shell contents lose water by diffusion and evaporation through the shell, the air space gradually increasing in volume and being, in some measure, an indication of the age of the egg. Bacteria can also gain access to the contents through the shell, and the longer the exposure the greater will be the contamination from this source. Since an egg submitted to preservation processes as soon as it has cooled (which is the most suitable time) can be retained for considerable periods without decomposition, whilst stale eggs similarly treated continue to deteriorate, it is probable that bacterial contamination is the primary cause of decomposition. Preservation with oil closes the pores of the shell with a film of oil which prevents ingress of bacteria. Silicate or lime solutions, which are both alkaline, may derive their preserving properties from their bactericidal action. On the other hand, they might mechanically close the pores of the shell. To investigate this point, tests have been carried out on the porosity of the shell of unpreserved and preserved eggs, and it has been shown that both silicate and lime solutions close the pores. This explains the well-known household experience that preserved eggs generally crack on being boiled. Such alteration in the porosity of the shell is characteristic of the three types of preservation under consideration. It is readily demonstrable and may be regarded as proof of preservation. Tests are described later for the characterisation of the method of preservation.

POROSITY OF THE SHELL.—Attempts were first made to obtain a quantitative measure of the degree of porosity by finding the quantity of water capable of being absorbed by the shell. Weighed pieces of shell were placed in a vessel which was then evacuated by means of a mercury pump. Water was introduced to cover the shell, the pressure restored, and the pieces quickly wiped and weighed. On account of the small quantity of water retained and its rapid evaporation, reliable results could not be obtained. A simple test was then devised to indicate whether or not the shell was porous. The principle of the test is to place a drop of dye solution on one side of the shell and to reduce the pressure on the other side to about half an atmosphere. With porous shells the dye is rapidly sucked through the shell over the whole of the exposed surface, whilst no colour passes through when the shell is non-porous. The apparatus used is shown in the diagram, but several other forms could equally well be used. It consists of a piece of glass tubing, about 5 cm. long and 5 mm. in internal diameter, which can be inserted in a rubber bung fitted to the end of a similar piece of glass tubing about 20 cm. long. The other end of the longer tube is connected with a small mercury reservoir by means of rubber tubing. In applying the test a small piece of shell is freed from the membranes on the inside by soaking in water for a few seconds, gently scraping with a knife, and rubbing between the thumb and fingers. It is then sealed to one end of the short piece of glass tubing by means of a tight joint of paraffin wax. The other end of the tube is inserted in the rubber bung after the reservoir has been so adjusted that the mercury stands approximately at the mark A, which is about 10 cm. below the egg shell. One or two drops of a 0·1 per cent. solution of methylene blue in 30 per cent. alcohol are placed on the top of the shell, and the mercury reservoir lowered until the mercury level stands at the mark B, about 20 cm. below the egg-shell. It is immaterial from the point of view of the success of the test whether the tube is attached to the outer or inner side of the shell, but, for convenience in holding the dye solution, it is better to attach it to the outer side. This can readily be done by placing the shell on a level surface, carefully resting the glass tube upon it, and painting molten paraffin wax on the joint with a small brush.



By means of this test over fifty new-laid eggs from different parts of the country, and over sixty eggs known to have been preserved, have been examined. Every new-laid egg showed colour penetrating the shell in 5 seconds or less, and in 30 seconds the whole surface was flooded. The penetration in the exposed circle, of about 5 mm. diameter, takes place at numerous spots—twenty or more—and the droplets of dye solution quickly unite. In the case of preserved eggs either no penetration took place after several minutes or else very slight colour was visible at an isolated spot or two. As a working test one minute was taken as the limit of time when the small tube was removed for examination. By gently warming the glass tube it can be removed from the shell, leaving the

wax-surrounded circle of shell intact. With several of the preserved eggs the test remained overnight under reduced pressure, and no trace of colour passed through the shell.

ALKALINITY OF THE SHELL.—Since both silicate and lime solutions are strongly alkaline, it was thought possible that eggs preserved in them might be differentiated from other eggs by tests for alkalinity, since, even if the shells were washed, traces of the preserving medium might remain. Ground egg-shells were tested with a universal indicator, and the shells of both preserved and unpreserved eggs reacted in practically the same way as well-washed precipitated calcium carbonate. Unopened eggs were soaked in warm water, and the *p*H of the poured-off liquid tested. This varied from 8 to 10 for both preserved and unpreserved eggs, and did not appear to be significant.

EXAMINATION FOR TYPE OF PRESERVATION.—(1) *Oiled Eggs.*—All the eggs of this type which could be obtained had been treated with oil, or with oil and wax mixtures. In every case sufficient of the preserving material remained to affect the "wetting" power of the shell. If a drop of water is placed on an unpreserved shell it wets the surface, and, when rubbed with a glass rod, the wetted film spreads. Silicated and limed eggs behave similarly. With oiled eggs the surface is not wetted, and the drop can be completely rolled over the shell surface. A drop of 95 per cent. alcohol remains as a globule on oiled eggs, whilst on other eggs it quickly spreads over the surface. Even if the eggs are well wiped with a dry cloth to remove as much surface oil as possible the effect remains. Hence it appears that if there is sufficient oil to seal the pores, its presence materially alters the interfacial tension and can be demonstrated.

By soaking oiled eggs in a suitable solvent a weighable quantity of the residual preserving medium can usually be obtained. Unopened unoiled eggs, washed with decalin, gave 2 to 3 mgrms. of soluble matter, whilst oiled eggs similarly treated yielded from 9 to 38 mgrms. Attention must here be called to the possibility of eggs becoming accidentally contaminated with grease. It may be necessary to examine several portions of the shell for porosity to exclude this possibility. Adventitious grease does not usually seal pores except in small patches, and by drawing a coloured solution through the shell from the inside to the outside it will ooze round particles of grease not actually in the pores.

Attempts have been made to employ an oil-soluble dye for distinguishing this type of preservation. A drop of a solution of the dye in strong alcohol was placed on the shell and allowed to evaporate. The shell was then placed in 50 per cent. alcohol to see how easily the stain was washed off. In some cases the oiled eggs had fixed the colour, but, owing to the conglobation of the alcoholic solution on their surfaces, the colour was always more concentrated than on unoiled eggs. In fact, the behaviour of the drop of alcoholic dye solution appeared more certain than the retention of colour after washing the stain with 50 per cent. alcohol. If sufficient of the oil remains to absorb dye, it shows by its surface action.

(2) *Silicated Eggs.*—For the characterisation of this type of preservation two lines of attack suggested themselves, namely, (*a*) the possible action of the alkaline silicate solution on the albuminous material in the shell basis, whereby the adsorption of dyes by the shell might be facilitated, and (*b*) the detection of silica.

With regard to (*a*) several dyeing tests were carried out, and it appeared that certain dyes were capable of being adsorbed to a much greater extent on silicated than on other shells. The intensive coloration appeared as a surface layer on the outside of the shell, whereas if the albuminous layers had been attacked it would have been expected that the dye would have penetrated below the surface. The most suitable dye for the purpose of this test was methylene blue, the same solution as that used for the porosity tests. A small portion of the shell, after removal of the adhering membranes, was boiled with the dye solution for about 5 seconds and then washed. Silicated shells give a deep indigo-blue colour on the outside of the shell, the inside being light blue. Other shells give the same light blue colour on the inside, but the outside is pale bluish with white eggs and greenish with brown eggs. The colour produced on the shell is due to a surface deposit of silica, which strongly adsorbs the dye. The same indigo-blue colour is produced by boiling precipitated silica with the dye solution, filtering and washing, and this may be used as a colour standard for comparing tests on egg-shells. Other useful dyes for giving distinguishable colour tests are malachite green and methyl violet.

(*b*) For the detection of silica, the shell was soaked in water, and the poured-off solution tested for soluble silica with molybdic acid. It was found most convenient to cover an unopened egg with water at about 40° C., and, after 15 minutes, to pour off the water and add 2 ml. of molybdic acid solution (2 grms. of ammonium molybdate and 40 ml. of *N* sulphuric acid to 100 ml.). The yellow colour produced after standing 15 minutes was matched by diluting 0·07 per cent. picric acid solution, 1 ml. of which in 50 ml. of water gives a colour approximately equal to that given by 1 mgrm. of silica. When either unpreserved, oiled, or limed eggs were so treated, the silica found never exceeded 0·2 mgrm. per 100 ml. of washing solution; with silicated eggs approximately 10 times this quantity was obtained. By repeating the soaking, silicated eggs gave further quantities of soluble silica, whilst other eggs gave negligible traces. Very dirty new-laid eggs gave no more silica than clean ones. The scrapings from two different chicken-runs were soaked in warm water and filtered. The filtrate tested with molybdic acid gave practically no colour.

These tests are sufficient to establish whether a preserved egg has been silicated or not.

(3) *Limed Eggs.*—The Board of Agriculture Leaflet No. 83, May, 1910, on the Preservation of Eggs, states that limed eggs can easily be recognised by the roughness of the shell. The 32 samples examined were very little different in texture from unpreserved eggs, and the reduction of the slight shininess or bloom usually present in unpreserved eggs was insufficient to render this distinction reliable.

Since the shell becomes non-porous by remaining in lime-water, it appears that the sealing agent must be calcium carbonate deposited there, and some means are required for differentiating between deposited carbonate and the material of the shell. For this purpose chemical tests seem useless. It was thought that a difference in the rate of solubility might be detectable, and the soluble lime was determined after soaking unopened eggs in water. As much was obtained from unpreserved and silicated eggs as from limed eggs.

The temperature of a hen is in the neighbourhood of 40° C., and the shell basis has been formed at this temperature. Any calcium carbonate deposited in the pores will have been produced at atmospheric temperatures which will almost certainly be below 20° C., and will generally be much lower, as care is taken in storing eggs in preserving solutions to keep the temperature as low as convenient. Consideration was therefore given to the possibility of distinguishing calcium carbonates formed at different temperatures. It is generally stated that when calcium carbonate is precipitated below 30° C., calcite is formed; above 70° C., aragonite is produced; between these temperatures a mixture of the two isomorphs is obtained. Johnston, Merwin and Williamson (*Amer. J. Sci.*, 1916, [IV], **41**, 473) state that at 60° C. another form, referred to as $\mu CaCO_3$, results. This has a specific gravity of 2·54, the respective values for calcite and aragonite being 2·71 and 2·88. The specific gravity of air-dried ground egg-shell has been found to be 2·50, a value which is, of course, dependent on the proportions of organic and mineral matter. Microscopic examination of egg-shells revealed no characteristic crystalline forms, and a careful scrutiny of the surface and of scrapings of several limed eggs showed no recognisable calcite crystals.

Various qualitative tests have been described for the differentiation of calcite and aragonite, *e.g.* the cobalt nitrate test of Meigen (*Centr. Min.*, 1901, 577), the ferrous sulphate test of Panebianco (*Z. Kryst.*, 1904, **40**, 288), and of Diesel (*Z. Kryst. Min.*, 1911, **49**, 250), the dyeing tests of Thugutt (*Chem. Zentrbl.*, 1910, ii, 1084). It is generally considered that such tests are to be interpreted very carefully before being regarded as specific for either of the isomorphous forms (see Johnston, Merwin and Williamson, *loc. cit.*). The tests might, however, have some value for the problem under consideration. Calcium carbonate was therefore prepared by passing carbon dioxide into lime-water at 40° C., and a second preparation was made in the same way at 15° C. Portions were tested by boiling with reagents for a short time, filtering through a Gooch crucible, and washing. Of the substances specified in the above papers, only Congo red gave a marked difference with the two carbonates. The one precipitated at 40° C. gave a bright orange-red colour, whilst that formed at 15° C. gave but a faint flesh-pink. Suitable conditions for applying the test were to employ a 0·1 per cent. aqueous solution of Congo red and to boil for about 5 seconds. The test was then applied to portions of egg-shells, and it was found that unpreserved eggs gave a good orange-red colour on both the outside and inside of the shell, of practically the same intensity as that obtained from the carbonate precipitated at 40° C. The colours produced on the

outside of white and brown eggs were almost identical, the depth of colour covering the normal differences in tint. Limed eggs gave the same colour as unpreserved eggs on the inside of the shell, but the outsides were considerably lighter. The colour varied from a very pale flesh colour to an orange-red and was sometimes irregularly distributed. The test has been applied to all the eggs available, including over 50 new-laid eggs and 32 limed eggs. The colours produced were assessed by comparison with a scale obtained by applying the test to calcium carbonate precipitated at 40° C., and diluting the washed and dried coloured product with undyed calcium carbonate; they were as follows:

Colour shade.	New-laid eggs.	Limed eggs.
Full	All	None
Three-quarters	—	12
Half	—	14
Quarter	—	6

The carbonate precipitated at 15° C. gave a colour about equal to the quarter standard.

Other dye solutions were tried, and similar results were obtained with alizarine S (0·1 per cent. aqueous solution) and the sodium salt of alizarine (0·1 grm. of alizarine and 5 ml. of $N/10$ sodium hydroxide solution to 100 ml.). With alizarine S both the outside and inside of unpreserved egg-shell were coloured purplish red to approximately the same extent; limed eggs gave a similar colour on the inside, but the outside was considerably lighter. In the case of the sodium salt of alizarine the unpreserved shell adsorbed much more colour on the outside than on the inside, the outside having a blackish lustre. This lustre effect was absent from the shells of limed eggs similarly treated, the outside being again deeper than the inside, but considerably lighter than the outside of unpreserved shell.

These colour tests have been applied to oiled and silicated eggs. All the oiled eggs reacted similarly to unpreserved eggs, except that with the sodium salt of alizarine the blackish lustre was only slightly visible. Silicated eggs, however, reacted like limed eggs, the film of deposited silica evidently protecting the basis of the shell.

The above-described dyeing tests do not, therefore, distinguish between limed and silicated eggs. But, as already indicated, the silicated eggs can be identified by other means. A non-porous egg which fails to absorb dyes when tested as described above, and gives no reactions for silica cannot be affirmed, definitely, to be limed, yet the statement can be made that the composition of the shell has been altered, and that it reacts in the same way as limed eggs and probably has been limed.

GENERAL METHOD OF EXAMINATION.—From the tests which have been developed the following procedure has been adopted for the examination of suspected eggs. With samples of less than one dozen eggs, at least half the

number are placed in separate 150 ml. beakers; with larger samples a representative number is taken. The eggs are covered with warm water at about 40° C., note being made of the surface behaviour as the water is poured on each egg. The water is maintained at about 40° C. for 10 to 15 minutes, and is then poured off, and to each solution is added 2 ml. of molybdic acid solution (2 grms. of ammonium molybdate and 40 ml. of *N* sulphuric acid to 100 ml.). After standing about 15 minutes the colour, if any, is matched with that given by adding picric acid solution (0·07 per cent.) to a similar quantity of water. If not more than about 0·2 ml. of picric acid solution is required, the dissolved silica is regarded as normal. Each egg is then broken or blown and the contents discarded. Portions of the shell, about ½ inch by ½ inch, are placed in water, and the egg and shell membranes removed by gentle scraping and rubbing. It may be mentioned that the egg membrane can generally be peeled off easily, but the shell membrane adheres closely to the shell. Two or three pieces of the shell from each egg are then tested for porosity. Complete absence of porosity or very slight porosity, in all the pieces tested is regarded as evidence of preservation. Further portions of each shell are then tested by boiling for about 5 seconds with (1) methylene blue solution (0·1 per cent.) and (2) Congo red solution (0·1 per cent.) and then washing. If care is taken to prevent overlapping, several pieces of shell may be boiled at the same time with either reagent, including pieces of unpreserved egg-shell used as controls. Other portions of shell are tested for surface action with a drop of strong alcohol.

According to the results obtained, further tests, as described above, may be tried, and other eggs from the sample may be tested.

SUMMARY.—(1) Unpreserved eggs are porous, whilst eggs preserved in oil, water-glass, or lime-water, are non-porous.

(2) Oiled eggs retain traces of oil in the pores, which give marked surface effects, but which do not prevent normal adsorption of dyes by the shell.

(3) Silicated eggs have a surface-film of silica which seals the pores, and which can be detected by extraction and by its behaviour towards certain dyes.

(4) Limed eggs have a surface-film of deposited calcium carbonate, which seals the pores and which reacts differently towards certain dyes from the normal calcium carbonate of the shell.

(5) A test for porosity of the shell, and a method for the examination of eggs suspected of being preserved are described.

I wish to thank the Government Chemist for permission to publish this paper.

GOVERNMENT LABORATORY,
CLEMENT'S INN PASSAGE, STRAND, W.C.2.

The Determination of Soluble Solids in Jams, etc.

A COMMUNICATION FROM THE BRITISH ASSOCIATION OF RESEARCH FOR THE COCOA, CHOCOLATE, SUGAR CONFECTIONERY AND JAM TRADES.—T. MACARA, F.I.C., *Director of Research.*

IN the Standards for Jams (ANALYST, 1930, **55**, 694) a minimum limit for soluble solids was included. It is exceedingly difficult to determine the true percentage of soluble solids, and the limit was, therefore, coupled up with a covering definition. This reads as follows:—

> "All jams, whether of First Quality or Second Quality shall contain not less than 68½ per cent. total soluble solids, by refractometer reading when cold, uncorrected for insoluble solids."*

It will be obvious at a glance that the solids determined in this manner may be 2 or 3 per cent. higher than the truth if the insoluble solids are high. There are other errors which, in some cases, cancel each other to a greater or less extent, for the reading is taken either direct in percentages of sucrose, or is converted into these from the refractive indices by means of tables or the corresponding factors. As the jam contains invert sugar, acids and salts, all having refractive indices differing from that of sucrose, the soluble solids determined in this manner may be 1 or 2 per cent. wide of the truth; *e.g.* invert sugar, when present in large proportions, gives a low result.

This method of determining the soluble solids, which was adopted by the Public Analysts' Committee and the Food Manufacturers' Committee jointly, has many advantages from the practical standpoint, and is more satisfactory to all concerned than leaving it open to all parties to select their own methods.

The advantages of this system may be summarised briefly. In the first place the refractometer has been used for a comparatively long time to control the composition of the jam at the time of manufacture, the determination of the true soluble solids by any other means taking much too long. Further, refractometers are in general use, and the taking of this reading is simple, compared with other methods. In this way both manufacturers and analysts will be using the same methods.

In this connection there is, however, one objection to the method, *viz.* that only a very small portion of the jam is tested, and, as jam is by no means a homogeneous mixture, results which do not correspond with that of the carefully averaged sample may readily be obtained.

For example, the surface layer of an old jam may give a reading as much as 8 per cent. or more above that of the bulk of the jam. If, in testing such a sample, care is not exercised to see that the whole sample is carefully mixed, wrong results may be obtained. The same remarks apply in a less degree to layers of the jam all the way down the jar. This effect is due to the fact that under normal storage conditions the evaporation of moisture from the surface is more rapid than its

* The "cold" temperature is taken as 20° C. in the laboratory of the Association.—T.M.

diffusion upwards from the lower portions. These remarks also apply to whole-fruit jams in which the pieces of fruit may have a different soluble solids content from that of the jelly portion.

This objection is easily overcome if the mixing of the sample is sufficiently thorough, all the whole fruit being well crushed, or, preferably, the entire sample being passed through a small mincer.

Refractometers are now made with scales giving both refractive indices and corresponding sugar percentages. Messrs. Bellingham and Stanley also make a butyro-refractometer which contains a scale showing both the usual numbers and the corresponding sugar percentages. As, however, these instruments are of a special type, the following table for use with the ordinary types will be found convenient in testing jams, syrups, etc.

The table has been prepared by C. L. Hinton, who has given the corresponding sugar values for both the refractive indices and butyro refractometer numbers. The butyro-refractometer numbers equivalent to the indices have been taken from the table given in E. R. Bolton's "Oils, Fats and Fatty Foods." The scale of sugar percentages from 61 to 75 is based on data arrived at experimentally by Hinton in the Association's laboratory. The higher percentages are based on Main's tables.

Hinton's scale between 61 and 66 agrees closely with that of Schönrock, but differs somewhat from Main's at the lower concentrations. Main's figures for the higher concentrations are believed to be reasonably reliable.

It should be noted that while temperature affects the reading, a difference of 5° C. only produces an error of 0·35 per cent. in the actual solids.

TABLE I.

n_D^{20}	Sugar. Per Cent.	Butyro ref. scale.	n_D^{20}	Sugar. Per Cent.	Butyro ref. scale.	n_D^{20}	Sugar. Per Cent.	Butyro ref. scale.
1·4440	61·1	28·3	1·4500	63·6	36·7	1·4560	66·2	45·2
2	·1	·6	2	·7	37·0	2	·3	·5
4	·2	·9	4	·8	·2	4	·4	·7
6	·3	29·2	6	·9	·5	6	·4	46·0
8	·4	·4	8	64·0	·8	8	·5	·3
50	·5	·7	10	·1	38·1	70	·6	·6
2	·6	30·0	2	·2	·3	2	·7	·9
4	·7	·3	4	·2	·6	4	·8	47·2
6	·7	·6	6	·3	·9	6	·9	·5
8	·8	·8	8	·4	39·2	8	·9	·7
60	·9	31·0	20	·5	·5	80	67·0	48·0
2	62·0	·4	2	·6	·7	2	·1	·3
4	·1	·6	4	·7	40·0	4	·2	·6
6	·2	·9	6	·8	·3	6	·3	·9
8	·2	32·2	8	·9	·6	8	·4	49·2
70	·3	·5	30	·9	·9	90	·4	·5
2	·4	·8	2	65·0	41·1	2	·5	·8
4	·5	33·0	4	·1	·4	4	·6	50·1
6	·6	·3	6	·2	·7	6	·7	·4
8	·7	·6	8	·3	42·0	8	·8	·7
80	·8	·9	40	·4	·3	1·4600	·8	51·0
2	·9	34·2	2	·4	·5	2	·9	·3
4	·9	·4	4	·5	·8	4	68·0	·6
6	63·0	·7	6	·6	43·1	6	·1	·9
8	·1	35·0	8	·7	·4	8	·2	52·2
90	·2	·3	50	·8	·7	10	·3	·5
2	·3	·6	2	·9	44·0	2	·3	·8
4	·4	·8	4	·9	·3	4	·4	53·1
6	·5	36·1	6	66·0	·6	6	·5	·4
8	·6	·4	8	·1	·9	8	·6	·7

TABLE I.—*continued.*

n_D^{20}	Sugar. Per Cent.	Butyro ref. scale.	n_D^{20}	Sugar Per Cent.	Butyro ref scale.	n_D^{20}	Sugar. Per Cent.	Butyro ref. scale.
1·4620	68·7	54·0	1·4720	72·8	69·5	1·4820	76·8	86·4
2	·8	·3	2	·9	·9	2	·9	·7
4	·9	·6	4	73·0	70·2	4	77·0	87·1
6	·9	55·0	6	·1	·5	6	·1	·5
8	69·0	·3	8	·1	·8	8	·1	·8
30	·1	·6	30	·2	71·1	30	·2	88·2
2	·2	·9	2	·3	·4	2	·3	·5
4	·3	56·2	4	·4	·8	4	·4	·9
6	·4	·5	6	·5	72·1	6	·4	89·2
8	·4	·8	8	·6	·4	8	·5	·6
40	·5	57·1	40	·6	·7	40	·6	90·0
2	·6	·4	2	·7	73·0	2	·7	·3
4	·7	·7	4	·8	·3	4	·8	·7
6	·8	58·0	6	·9	·7	6	·8	91·1
8	·9	·3	8	74·0	74·0	8	·9	·4
50	·9	·6	50	·0	·3	50	78·0	·8
2	70·0	·9	2	·1	·6	2	·1	92·1
4	·1	59·2	4	·2	75·0	4	·2	·5
6	·2	·5	6	·3	·3	6	·2	·9
8	·3	·8	8	·3	·6	8	·3	93·2
60	·4	60·2	60	·4	76·0	60	·4	·6
2	·4	·5	2	·5	·3	2	·5	94·0
4	·5	·8	4	·6	·7	4	·6	·3
6	·6	61·1	6	·7	77·0	6	·6	·7
8	·7	·4	8	·7	·3	8	·7	95·0
70	·8	·7	70	·8	·7	70	·8	·4
2	·9	62·0	2	·9	78·1	2	·9	·8
4	·9	·4	4	75·0	·4	4	·9	96·1
6	71·0	·6	6	·1	·7	6	79·0	·5
8	·1	·9	8	·1	79·1	8	·1	·9
80	·2	63·2	80	·2	·4	80	·2	97·2
2	·3	·5	2	·3	·8	2	·3	·6
4	·3	·8	4	·4	80·1	4	·3	98·0
6	·4	64·2	6	·5	·5	6	·4	·3
8	·5	·5	8	·6	·8	8	·5	·7
90	·6	·8	90	·6	81·2	90	·6	99·1
2	·7	65·1	2	·7	·5	2	·7	·4
4	·8	·4	4	·8	·9	4	·7	·8
6	·8	·7	6	·9	82·2	6	·8	
8	·9	66·1	8	76·0	·5	8	·9	
1·4700	72·0	·4	1·4800	·0	·9	1·4900	80·0	
2	·1	·7	2	·1	83·2	2	·0	
4	·2	67·0	4	·2	·6	4	·1	
6	·2	·3	6	·3	·9	6	·2	
8	·3	·7	8	·3	84·3	8	·3	
10	·4	68·0	10	·4	·6	10	·3	
2	·5	·3	2	·5	85·0	2	·4	
4	·6	·6	4	·6	·3	4	·5	
6	·7	·9	6	·6	·7	6	·6	
8	·7	69·2	8	·7	86·0	8	·6	

THE DETERMINATION OF "TRUE" SOLUBLE SOLIDS.

Although the percentage of true soluble solids is not required in the analysis of jams in connection with the maintenance of the Standards, this figure may sometimes be of interest to analysts. In such cases, the following notes on the subject may be helpful:

The determination of the solids by drying methods does not yield satisfactory results, as the acids present in the jam not only invert the sugar, but also tend to

decompose it. Further, the sugars, particularly laevulose, do not readily part with the last traces of moisture.

Distillation methods, employing volatile non-miscible liquids, would probably be equally unsuitable, for the same reasons.

The best methods would appear to be indirect ones, depending on either the refraction of the jam itself or on that of a solution, or on the gravity of this solution, using suitable factors for calculating the percentage of sugar present, and making the necessary corrections for the effects of the insoluble solids, acids, etc., in the jam. These methods have been worked out in great detail in the Association's laboratories, and the tables given below have been computed from data arrived at experimentally.

By Direct Refractometer Reading.—As already pointed out, there are difficulties in obtaining from a minute portion of the sample a reading which will give a result of the desired accuracy and truly representative of the bulk of the jam. With the necessary care, this can, however, be attained. The reading so obtained is then converted into percentage of sugar by means of Table I, and afterwards corrected for the errors due to the different refractive values of invert sugar, glucose (if present) and acid; this result is then corrected for the effect of the insoluble solids.

In order to simplify these calculations a table is given below from which the corrections for invert sugar, glucose and acid can be readily computed, and an example is given to illustrate the method.

Table II.

Corrections for Invert Sugar, Glucose Solids and Acids to be used in Calculating Total Soluble Solids from the direct reading on the Refractometer.

Substance.	Percentage in sample.	Correction.
Invert sugar	20	+0·45
	40	+0·90
	60	+1·35
Glucose solids	20	−0·25
	40	−0·5
Citric acid	1	+0·1
	2	+0·2
	3	+0·3

Example I.—A sample of black currant jam gave the following results on analysis:

Insoluble solids	2·0 per cent.
Invert sugar	31·0 ,, ,,
Acidity as citric acid ..	0·95 ,, ,,
Direct refractometer reading	$n_D^{20} = 1{\cdot}4673$

Calculation.—n_D^{20}, 1·4673, corresponding with 70·9 soluble solids as sucrose.

Correction for invert sugar,	+	0·7
,, ,, acid,	+	0·1
Total		71·7

This is corrected for insoluble substances thus:

$$\frac{71{\cdot}7 \times (100 - 2{\cdot}0)}{100} = 70{\cdot}3 \text{ per cent.}$$

DETERMINATION OF SOLUBLE SOLIDS BY SPECIFIC GRAVITY METHOD.—In this case a 20 per cent. solution of the jam is made by dissolving 50 (or 100) grms. of the jam.* The solution and fibre are transferred to a 250 (or 500) ml. flask and made to volume at 20° C. The solution is next filtered through muslin or a fine sieve and the gravity determined as usual at 20° C.

The percentage of solids in solution is then calculated as sucrose, using the factor 3·845, which is that which has been found to be correct for sugar solutions of this concentration and at 20° C. The solids are then calculated to percentage in original sample and corrected for invert sugar, etc., using the corrections given in Table III, and afterwards for volume of insoluble, as shown in the following example:

TABLE III.

Corrections to be used where solids are determined in 20 per cent. solution at 20° C. and the solution factors 3·845 and 1·428 are used for calculating the solids obtained by gravity and dipping refractometer respectively.

Substance.	Apparent percentage in sample.	Correction on solids: by sp. gr. at 20°/20° C.	Correction on solids: by dipping refractometer n_D^{20}.
Invert sugar	20	−0·07	+0·22
	40	−0·14	+0·44
	60	−0·21	+0·66
Glucose solids	20	−0·34	−0·46
	40	−0·68	−0·92
Citric acid	1	−0·09	+0·12
	2	−0·18	+0·24
	3	−0·27	+0·36

Example II.—Black currant jam as in I.

Sp. gr. of 20 per cent. solution = 1·05423

Soluble solids as sucrose $\frac{54{\cdot}23}{3{\cdot}845} \times \frac{100}{20}$ = 70·5 per cent.

Correction for invert sugar, = − 0·1 ,, ,,

,, ,, acid, = − 0·1 ,, ,,

Total sugar 70·3

This has to be corrected for the volume of the insoluble matter in the solution, and for this purpose it has been found to be satisfactory to assume that its gravity is 1. Actually the gravities of the various insoluble matters of fruit vary from about 1·4 to 1·8, but there appears to be a compensating error which is thought to be due to adsorption of the sugars. In any case the error, if any, is small. In the present instance the percentage corrected in this way is:

$$\frac{70{\cdot}3 \times (100 - 0{\cdot}4)}{100} = 70{\cdot}0 \text{ (}cf.\text{ result in Example I.)}$$

DETERMINATION BY MEANS OF THE DIPPING REFRACTOMETER.—The 20 per cent. solution is prepared as for the gravity and the reading is taken at 20° C. This instrument gives more exact readings than does the Abbé instrument. The reading is, however, on an arbitrary scale, which has then to be converted to

* Care should be taken when dissolving the jam to keep the solution at a sufficiently low temperature to prevent inversion of the sugar.

indices. A table for this purpose is given in Leach's "Food Inspection and Analysis" (Fourth edition, p. 102). The refractive index so obtained is then converted to sugar percentage by deducting the refractive index of water, multiplying by 1000, dividing by the solution factor 1·428, which has been found to be the appropriate one for sugar solutions of this concentration and temperature, and, finally, bringing to percentage in original sample.

The necessary corrections are made as before (see fourth column, Table III).

Example III.—Black currant jam (analysis as in Example I).

n_D^{20} of 20 per cent. solution = 1·35302.

Soluble solids as sucrose:

$$\frac{(1{\cdot}35302 - 1{\cdot}3330) \times 1000}{1{\cdot}428} \times \frac{100}{20} = 70{\cdot}1$$

Correction for invert sugar	+	0·3
,, ,, acid	+	0·1
Total		70·5

Correction for insoluble substances as in Gravity Method:

$$\frac{70{\cdot}5\ (100 - 0{\cdot}4)}{100} = 70{\cdot}2.$$

If the three results are compared it will be noted that there is a maximum difference of 0·3. This is probably due mainly to the different effects of the salts and possibly of other substances in solution on the refractive index and gravity respectively. It is, therefore, usual to take the mean of the results obtained by the two methods, particularly where the refractive index has been taken by the dipping refractometer.

Some jams show greater differences between the results obtained by the two methods than others. Black currant is one of the worst in this respect, probably owing to the fact that it contains more salts and that it is more highly coloured than most of the others. Results for jam such as strawberry and apple frequently agree very closely, *i.e.* within 0·1 per cent.

Where it is desired to obtain approximately the true percentage of soluble solids, when the percentages of invert sugar and acid are unknown, the gravity method will give the best results, *i.e.* without applying the necessary corrections for these ingredients. A glance at the tables of corrections will show that these are smallest when using this method, and, in the case of the example, would have made an error of 0·2 at most, provided the correction for insoluble solids was made.

Errata.—In the May issue, p. 296, l. 15, *for* "0·2 mgrm." *read* "0·2 grm."

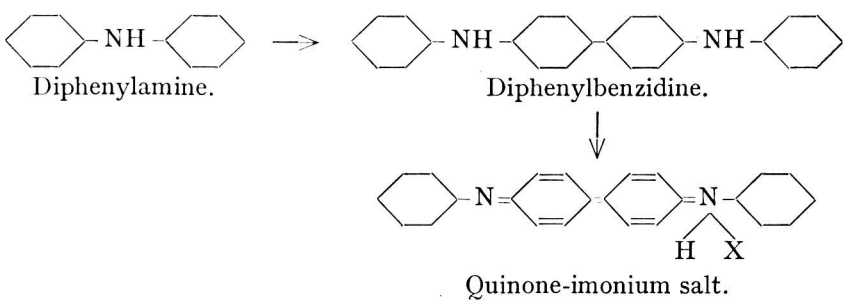
The Detection of Benzoic Acid, p. 303: The legends beneath the lower block should be reversed. "C" represents silver salicylate; "D" silver benzoate.

Notes.

The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

THE NITRATE TEST FOR THE DETECTION OF ADDED WATER IN MILK.

In several communications which have appeared from time to time in The Analyst, the use of diphenylamine is recommended as a test for nitrates in milk. (G. D. Elsdon and J. A. L. Sutcliffe, Analyst, 1913, **38**, 450; G. D. Elsdon and P. Smith, Analyst, 1922, **47**, 18; A. F. Lerrigo, Analyst, 1931, 433; D. R. Wood, E. T. Illing and A. E. Fletcher, Analyst, 1931, 248.) This test is more sensitive if diphenylbenzidine be substituted for diphenylamine. F. Kehrmann and St. Micewicz (*Ber.*, 1912, **45**, 2641) showed that the blue compound formed by the action of nitric acid on diphenylamine is a quinone-imonium salt of diphenylbenzidine. The action takes place in two stages, firstly, the oxidation of diphenylamine to diphenylbenzidine; and secondly, the oxidation of diphenylbenzidine to a quinone-imonium salt:



Diphenylamine. Diphenylbenzidine.

Quinone-imonium salt.

The first stage of the oxidation is effected at the expense of some of the nitrate present. If the milk contains only traces of nitrate, or if too much diphenylamine be used, it may happen that no nitrate is left to effect the second stage of oxidation and to produce the blue colour.

The preliminary stage of oxidation can be avoided by using ready-made diphenyl-benzidine, in which case the whole of the nitrate present is available for the second stage of oxidation, and the test becomes much more delicate and certain (*cf.* E. A. Letts and F. W. Rea, *J. Chem. Soc.*, 1914, **105**, 1157; L. Smith, *Z. anal. Chem.*, 1917, **56**, 28).

In my experience diphenylamine cannot be relied upon to detect nitrate in concentrations less than one part per million, but with diphenyl-benzidine a good gradation of colour is obtained with amounts of nitrate ranging from 0·1 to 1 part per million.

Diphenyl-benzidine is readily prepared from diphenylamine by Marqueyrol and H. Muraour's method (*Bull. Soc. Chim.*, 1914, [iv], **15**, 186). This method is not described in detail in the abstracts available. Eight grms. of diphenylamine, dissolved in 20 c.c. of glacial acetic acid, are poured into a mixture of 50 c.c. of

concentrated sulphuric acid and 150 c.c. of water which has previously been cooled to room temperature. To this solution is added, in small portions at a time, with shaking, a solution of 4·7 grms. of sodium dichromate in a mixture of 200 c.c. of water and 29 c.c. of dilute sulphuric acid (1 vol. H_2SO_4 + 4 vols. water). The solution becomes deep blue and a precipitate is formed, the temperature rising slightly. The liquid is shaken for a few minutes and then poured into a 2-litre flask containing 100 c.c. of a strong solution of sodium bisulphite (sp. gr. about 1·23) and 400 c.c. of water. A voluminous dark green precipitate is formed. The mixture is heated on a steam bath until the precipitate turns brown (about 60° to 70° C.). It is then allowed to cool and is filtered. The precipitate is washed with water and then with alcohol to remove unchanged diphenylamine, dried and crystallised from hot toluene. The yield of diphenyl-benzidine is about 60 per cent. of the theoretical amount. The product is more or less grey in colour, but may be used without further purification for the nitrate test. The technique of this test is the same as when diphenylamine is used.

G. W. MONIER-WILLIAMS.

Official Appointments.

THE Minister of Agriculture and Fisheries has confirmed the following appointments:

ROWLAND H. ELLIS, F.I.C., as Agricultural Analyst for the County of Herefordshire (May 9, 1931).

ERIC VOELCKER, A.R.C.S., F.I.C., as Deputy Agricultural Analyst for the County of Northumberland (May 9, 1931).

Legal Notes.

Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.

"CREAM" IN CONFECTIONERY.

J. LYONS & CO. *v.* KEATING.

ON February 6th the High Court remitted this case to the Swindon justices for them to consider whether the articles in question were sold as substances purporting to be cream as defined in the Artificial Cream Act, 1926 (ANALYST, 1931, 253). After a further hearing on March 9th, 1931, the justices found that the articles were sold as substances purporting to be cream as defined by the Act, and that there was evidence to support such a finding. An appeal against this finding was heard in the King's Bench Division (before the Lord Chief Justice, Mr. Justice Avory and Mr. Justice Charles) on May 7th, 1931. Sir John Simon, K.C., Mr. Roland Oliver, K.C., and Mr. C. Salmon appeared for the appellants, and Mr. G. D. Roberts and Mr. Elam for the respondents.

The Lord Chief Justice (Lord Hewart) said that he had come to the conclusion that the appeal ought to be allowed. When he looked at the title and the contents of the statute he thought that it was tolerably plain that the statute was dealing with the sale of cream or artificial cream *simpliciter*. The words were a little curious, but what was being considered and dealt with was a substance purporting to "be" cream or artificial cream; not "a substance purporting to contain" cream. If the Act had meant the latter, it would have been easy to say "a substance or article purporting to be or to contain cream." Whether that construction was what the Legislature meant to express he did not know, but it was what the Legislature had expressed. It might be very unfortunate, and, in some cases, entirely misleading, that composite articles should be sold under names including the word cream. But such gaps could only be filled by the Legislature and not by that Court. He thought that the argument of the appellants that the Act applied only to cream as a separate commodity was right. The question whether it was desirable that the word cream should be applied to confectionery of that sort might well receive attention; for nothing could be more misleading than the titles given to the articles in this case.

Mr. Justice Avory and Mr. Justice Charles gave judgment to the same effect, and the appeal was allowed.

ALLEGED DERMATITIS FROM METAL.

Sharp *v.* Salmon & Gluckstein.

On May 12 the plaintiff, a consulting surgeon, brought an action in the King's Bench Divisional Court (Mr. Justice Horridge) for damages for personal injuries alleged to have been caused by a wrist watch sold to him by the defendants.

Mr. Kingbury, for the plaintiff, said that after the watch had been worn for a few days on the wrist of the plaintiff there was intense irritation, and a rash spread up his arm, over the left side of his neck and across his brow.

The back of the watch, which was stamped "pure nickel," had been analysed, and found to be composed of 55 per cent. of copper, 13 per cent. of nickel, 31 per cent. of zinc, with traces of lead and tin, but no antimony. Far from being pure nickel, it was a variety of German silver, and was distinctly radioactive.

Mr. Hilbery, for the defence, suggested that the plaintiff had worn this watch tightly on his wrist and that this had set up the irritation.

Dr. Knowsley Sibley said that the plaintiff on June 18, 1929, was suffering from diffused dermatitis. In view of the analysis of the watch case, he was of opinion that the metal itself could not cause the dermatitis, which he attributed to pressure and sweating under the watch. He did not think that there would be enough radio-activity at the back of the watch to cause the trouble.

At the close of the evidence for the plaintiff the jury stopped the case, expressing the view that the watch was reasonably fit for ordinary use, and that the skin irritation was not caused by any defect of the watch, but by the wearing of it.

Judgment was entered for the defendants, with costs.

CHEESE SANDWICHES.

On April 23rd a firm was summoned at the North London Police Court for selling cheese sandwiches, the fat in the cheese of which contained 67 per cent. of fat other than milk fat.

Mr. Robertson, for the Islington Borough Council, said that the article between two biscuits making the sandwich was "margarine cheese," whereas cheese was defined by the Act as "the substance usually known as cheese, containing no fat derived otherwise than from milk."

The Magistrate dismissed the summons under the Probation of Offenders Act, but said that it would be a very much more serious matter if, after these proceedings, there should be any further breach of the law.

Government of Bihar and Orissa.

ANNUAL REPORT OF THE CHEMICAL ANALYST FOR THE YEAR 1930.

DURING the year there were examined 1527 samples of foods, drugs and waters received from 20 districts of 21 districts of the province.

GHEE.—Of 353 samples of ghee examined, 83 were samples of pure cow and buffalo ghee prepared in the laboratory by the native processes, with the object of testing the range of the Reichert–Wollny value and of the butyro-refractometer reading, the milk samples (from the individual cow or buffalo) being obtained through reliable officers from various parts of the province. The butyro-refractometer reading ranged from 40° to 46°, and the Reichert–Wollny value of buffalo ghee ranged from 41·2 to 26, and that of cow ghee from 35·4 to 15·5.

RAPE OIL AND MUSTARD OIL.—Of 237 samples examined, 95 were found to be adulterated or below the standard. The usual adulterants are linseed oil and niger-seed oil.

SWEETS.—Forty-six samples of sweets were examined, with a view to discover whether they were made with pure ghee or pure rape oil. Of 46 samples, 28 were found to have been from ghee or oil which was adulterated or below standard.

TEA.—Thirty-one samples of tea were examined, of which 27 were found to be adulterated or below standard.

MISCELLANEOUS.—Four samples of rice and 2 samples of pulse were examined in order to test their fitness for human consumption. These grains were preserved in tin and mercury amalgam. Two samples were found to be contaminated with mercury and tin amalgam, with lead as impurity.

J. C. DAS.

General Medical Council.

PHARMACOPOEIA COMMISSION: REPORTS OF SUB-COMMITTEES.

THE Pharmacopoeia Commission have published the Report of the Pharmaceutical Chemistry Sub-Committee and the Report of the Cod-liver Oil Colour Test Sub-Committee,* and invite helpful criticisms and comments on the suggested standards and tests.

* The booklet containing these reports may be obtained from the Office of the Pharmacopoeia Commission, 44, Hallam St., Portland Place, W.1. Price 3/-. The Cod-liver Oil Colour Test Report will be published *in extenso* in the July issue of THE ANALYST.—EDITOR.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Determination of the Calcium, Magnesium and Acid-Soluble Phosphorus of Milk by means of Trichloroacetic Acid Filtrates. G. P. Sanders. (*J. Biol. Chem.*, 1931, **90**, 747–756.)—A procedure is described for the determination of calcium, magnesium and acid-soluble phosphorus in cow's milk without ashing; it involves the trichloroacetic acid filtrate method. The elimination of the ashing method effects a saving of at least one working day in the preparation of the samples. Data are presented to show the accuracy of the method, as compared with the ashing method. It was first decided to eliminate the addition of water, in order to try to shorten the procedure and increase the degree of accuracy. A study was made of the use of equal parts of milk and acid solution. The calcium content of normal cow's milk was determined by the ashing method and the trichloroacetic acid method. One part of 10 per cent. acid solution was added to 1 part of milk. When skim milk was used, the filtration method produced results which were usually slightly high for calcium, not greater than 1·5 per cent.; when whole milk was used, the positive error was as great as 4·03 per cent. The results indicated an increased content of calcium in skim milk as compared with the same milk before skimming, and this increase corresponded closely with the percentage of fat in the whole milk. Finally, the use of 4 parts of 10 per cent. trichloroacetic acid solution with 1 part of milk was found to be most effective in the preparation of the protein-free filtrates for mineral determinations. A high degree of accuracy was attained in determinations of calcium and magnesium. In a number of milk samples, 68·4 to 81·9 per cent. of the phosphorus was found to be acid-soluble. There was no definite correlation between the amount of acid-insoluble phosphorus and the amount of casein; in general, however, milk high in casein was high in acid-insoluble phosphorus. The procedure should be of value in the study of phosphorus combinations in milk, with particular reference to casein and lipid phosphorus. The technique is as follows:—*Trichloroacetic Acid Filtrate Method.*—Twenty c.c. samples of cow's milk are pipetted into each of two 100 c.c. volumetric flasks, the pipette and flasks having been accurately calibrated. The flasks are filled to the 100 c.c. mark with 10 per cent. trichloroacetic acid solution, the acid being added slowly and the flask rotated constantly. The contents are thoroughly mixed and left stoppered for 30 minutes with frequent shaking. They are then filtered through dry 15 cm. acid-washed filter paper; the funnel is covered with a watch-glass during the filtration. From each sample 50 c.c. of the filtrate (corresponding with 10 c.c. of milk) are taken for the mineral determination. *Calcium.*—Calcium analyses are carried out by the method of Meigs, Blatherwick and Cary (*J. Biol. Chem.*, 1919, **37**, 1), which is a modification of the McCrudden (*J. Biol.*

Chem., 1911–12, **10**, 187) method. Calcium is determined as calcium oxalate, by permanganate titration. The precipitation of the calcium oxalate may be accelerated by heating, for there is no appreciable amount of heat-coagulable protein present to cause interference in the filtration and washing of the crystals when the 4:1 ratio of acid and milk is used. *Magnesium.*—Owing to the small amount of magnesium which occurs in milk, 40 c.c. samples of milk are made up to 200 c.c. volumes with 10 per cent. trichloroacetic acid solution, and, after filtering, 150 c.c. aliquot portions, corresponding to 30 c.c. of milk, are used. Magnesium determinations are carried out by Method II of the Association of Official Agricultural Chemists ((11), p. 29); in the analyses of the trichloroacetic acid filtrates the first addition of alcohol is omitted. *Acid-soluble Phosphorus.*—The samples are prepared as described for calcium, with the use of trichloroacetic acid as the protein precipitant and mineral solvent. Phosphorus analyses in the filtrate are carried out by the gravimetric procedure of the Association of Official Agricultural Chemists ((11), p. 3). Trichloroacetic acid solution added to milk produces a much coarser precipitate than does the mixture of sodium tungstate and sulphuric acid of Folin and Wu; the trichloroacetic acid mixture filters much more rapidly and yields a considerably larger amount of filtrate in proportion to the amount of milk used.

P. H. P.

Cause of Beet Odour and Taste in Milk and Butter. P. Post. (*Z. Unters. Lebensm.*, 1931, **61**, 171–174.)—The odour and taste of beet developed during October and November in milk and butter from districts where beet sugar is grown are not of bacterial origin, since they occur in milk obtained under conditions of controlled cleanliness to the same extent as in that obtained in the usual way. They are characteristic of cattle fed on beet-heads and leaves, and may be simulated by the addition of 10 mgrms. of trimethylamine hydrochloride to 100 grms. of milk. The trimethylamine (which probably originates from betaine) was separated from the tainted milk by distillation in the presence of lime-water, into hydrochloric acid; the distillate was evaporated, the residue extracted with absolute alcohol, the extract evaporated, and the residue examined. Tests for trimethylamine hydrochloride which are not given by ammonium chloride are: (*a*) the odour of trimethylamine on addition of sodium hydroxide, (*b*) a yellow precipitate with a solution of iodine in potassium iodide solution; (*c*) a white precipitate obtained from the solid salt with mercury potassium iodide solution. All gave positive results with 200 c.c. of the tainted and treated milks, and negative results with normal milk. It is considered impracticable to forbid the use of beet-fodder, though propaganda might restrict its use. As remedies, warming the milk under reduced pressure, and extraction of the butter first with water acidified with lactic or tartaric acid, and then with fresh water, have yielded promising results in the laboratory.

J. G.

Some Properties of Honey Colloids and the Removal of Colloids from Honey by Bentonite. R. E. Lothrop and H. S. Paine. (*Ind. Eng. Chem.*, 1931, **23**, 328–332.)—The amount of colloid substances present in honey varies

between about 0·1 per cent. for light coloured honey and 1 per cent. for dark (buckwheat) honey, as determined by ultra-filtration through standardised collodion membranes. These colloid constituents appear to influence such properties as colour, flavour, caramelisation point and crystallisation of the honey. When the *p*H value of honey is adjusted to 4·3, maximum flocculation of the colloids takes place. The flocculation can be produced by the addition of appropriate quantities of bentonite, a colloidal clay, the particles of which are negatively charged. The action is one of mutual flocculation of oppositely charged colloids, and results in a brilliantly clear honey which is lighter than the original in colour. W. P. S.

Organic Acids in Honey. E. K. Nelson and H. H. Mottern. (*Ind. Eng. Chem.*, 1931, **23**, 335–336.)—The quantity of total volatile acids in fifteen samples of different honeys varied from 0·011 to 0·051 per cent., and consisted mainly of a mixture of formic and acetic acids. Sage honey contained the largest amount of acetic acid (0·046 per cent.) and tulip honey the largest amount of formic acid (0·024 per cent.). Citric acid and malic acid were present in all the samples, and succinic acid was detected in the samples having a relative high acidity, namely, sourwood, cotton and tulip honeys. W. P. S.

Detection, Determination and Occurrence of Butyric Acid in Foodstuffs. J. Grossfeld and F. Battay. (*Z. Unters. Lebensm.*, 1931, **61**, 129–161.)—Butyric acid in the presence of small amounts of acetic acid may be detected by the odour of the solution obtained on distillation, after the removal of other substances by the action of alkaline potassium permanganate and reduction of the latter in turn by means of acid ferrous sulphate (*vide infra*). The limiting amounts detectable are 1:12,500 in pure aqueous solution, and 1:10,800 in admixture with 0·6 per cent. of acetic acid. Butyric acid may be determined by the method described in detail for wine (see following abstract), the conditions for which have been established as a result of a theoretical consideration of the distribution-coefficient of butyric and caproic acids between water and xylene or petroleum spirit, and of the volatilities of the different fatty acids on distillation. The method depends on the separation of capric acid and higher fatty acids by extraction with petroleum spirit, controlled oxidation of amino acids and residual acids other than butyric and acetic acids with alkaline permanganate, followed by distillation of the butyric and some of the acetic acid, formation of their potassium salts and determination of the percentage of potassium in the residue after evaporation (*A* grms.) by the perchlorate method. Then *B* (percentage of butyric acid in the residue) = 2·223 (141·23 − *k*), where *k* is the perchlorate value (*cf.* Grossfeld, ANALYST, 1930, **55**, 138), *i.e.* the percentage of potassium perchlorate from the mixed potassium salts. A table gives the corresponding values of *B* for values of *k* between 109·83 and 141·23. Then *AB*/100 gives the butyric acid in grms. It was found that for reproducible and rapid working it was sufficient to use a simple distillation apparatus under the prescribed conditions (*loc. cit.*), and to apply a correction for all losses (*cf.* Wiegner and Magasanik, ANALYST, 1920,

45, 24). The distillation-curve of butyric acid from ordinary aqueous solution indicates an almost linear relationship between the percentage in the distillate and the percentage volume distilled. This rate is less for the simple distillation of acetic acid. Distillation of 40 c.c. from 50 c.c. of solution containing all the necessary reagents, as in the prescribed conditions (*vide infra*), is rapid and reproducible, though an error is involved if the acetic acid present is in great excess over the butyric acid. The distillation curve, in the case of butter-fat, is very similar to that of pure butyric acid. For the determination of k, 60 per cent. perchloric acid is preferable to the usual 20 per cent., the loss by solution of potassium perchlorate being only 0·04 compared with 0·13 mgrm. per c.c. This method is preferable to titration of distillate described below. The method is applied to the following samples, for which the percentage butyric acid contents are given:—Milk (sweet 0·004, sour 0·008); cheese (Limburger 1·340, Harzer, 0·142; rindless Emmenthaler 0·066, Swiss 0·356); meat (beef 0·036, pig 0·010, mutton 0·009); sauerkraut, 0·071; pickled cucumber, 0·023 (liquor 0·022); sultanas and currants, 0. The following results are in grms. per litre:—Wine labelled "Malaga," 0·110; best Samos, 0; poor sweet wine labelled "Samos," 0·203; dry red wine, 0; (*cf.* following abstract); wine distillate, 0·087; brandy, 0·234; beer (light 0·048, dark 0·081); pressed yeast, 0·019. J. G.

Adulteration of Sweet Wine and its Detection by Determination of the Lower Fatty Acids (Butyric Acid). A. Miermeister and F. Battay. (*Z. Unters. Lebensm.*, 1931, **61**, 161–171.)—As a qualitative test for carob (St. John's bread) in currant or raisin wine, 50 c.c. of wine are evaporated to 10 c.c. on the water-bath with 1 c.c. of 50 per cent. potassium hydroxide solution, and the residue acidified with 25 per cent. phosphoric acid and diluted to 200 c.c. From this 50 c.c. are then distilled, 5 c.c. of an alkaline 1 per cent. solution of potassium permanganate added to the distillate and, after 2 hours, 5 c.c. of acid ferrous sulphate solution (*vide infra*), and the mixture redistilled. If 1, 2 and 7 c.c. fractions are collected, the carob odour (*iso*-butyric acid) can be detected in the 2 c.c. fraction. If the test is positive, butyric acid should be determined by the method of Grossfeld and Battay (*cf.* preceding abstract). The wine (200 c.c.) is distilled with phosphoric acid, 160 c.c. collected, neutralised with N potassium hydroxide solution, 4 c.c. of a 50 per cent. potassium hydroxide solution added in excess, and the mixture heated for 30 minutes under a reflux condenser. After acidification with phosphoric acid the solution is diluted to 200 c.c., and 160 c.c. shaken with 40 c.c. of petroleum spirit (b.pt. 70° C.) for 2 minutes. The aqueous phase is neutralised with N sodium hydroxide solution, an excess of 1 c.c. added, the liquid evaporated, and the residue extracted with 10 c.c. of water. Potassium permanganate (20 c.c. of a 1 per cent. solution) is added, followed after 24 hours by 20 c.c. of a mixture containing 200 grms. of ferrous sulphate and 50 c.c. of sulphuric acid per litre, to destroy any excess. The mixture is then distilled (*cf.* preceding abstract), 40 c.c. collected in a 100 c.c. flask (previously dried at 120° C. and weighed), and titrated exactly to the end-point (X c.c.) with 0·1 N potassium hydroxide solution in the

presence of 3 drops of 0·25 per cent. phenolphthalein solution. Then $0{\cdot}013856X$ gives the potassium perchlorate equivalent (C). The liquid is then evaporated, the residue dried at 105° to 140° C. and weighed (A), when $k=100C/A$. In accurate work k may be determined from the weight of potassium perchlorate produced by treatment with perchloric acid (*loc. cit.*). The butyric acid content is thence found as described, and corrected (by the factor 1·15) for extraction, distillation and other losses. The mean molecular weight of the "middle" fatty-acids (which proved to be absent from sweet wines) may be found by washing the petroleum spirit extract with 20 c.c. of water, followed by 10 c.c. of 0·1 *N* potassium hydroxide solution, and by 35 c.c. of water. The alkaline extract is distilled with 5 c.c. of 25 per cent. phosphoric acid, and the distillate treated as already described, to determine k. No butyric acid was obtained from currants or raisins or from genuine sweet wines made from them. Of 13 purchased samples of Samos or Grecian sweet dessert wines, 2 contained no butyric acid, 5 contained 30 to 60 mgrms. per litre, and 6 (having distinct carob character) 80 to 250 mgrms. per litre. The ultra-violet lamp, which is considered useful only for sorting purposes (*e.g.* when the wine contains more than about 35 per cent. of carob), gives an intense luminescence with carob wine, but only a feeble colour with pure raisin wine. The fluorescence was simulated by ethyl butyrate, but not by butyric acid or by *iso*-butyric acid or its ethyl ester (*cf.* Berg and Stockert, *id.*, 1929, **57**, 448; Kickton and Berg, *id.*, 1928, **56**, 397). J. G.

Detection of Fruit Wine in Grape Wine by the Sorbitol Method. M. Klostermann and W. Fachmann. (*Z. Unters. Lebensm.*, 1931, **61**, 100–103.)—The wine (100 c.c.) is treated with 5 grms. of animal charcoal, filtered into a 300 c.c. flask, and evaporated on the water-bath under reduced pressure till viscous. Dextrin and pectins are then eliminated by extraction with 150 c.c. of absolute methyl alcohol, and amino and organic acids precipitated in the extract by 1 c.c. of lead acetate, the precipitate being collected on a porcelain suction-filter and washed with methyl alcohol. Excess of lead is removed from the filtrate by passage of a minimum volume of hydrogen sulphide, and the filtered solution evaporated under reduced pressure to 20 c.c. in a large test-tube provided with a capillary air-inlet to prevent foaming. Three c.c. of pyridine and 3 times the volume of acetic anhydride are then added, and, after 45 minutes on the water-bath, 20 c.c. of water are added, the acidity reduced by addition of sodium carbonate (the solution saturated with sodium chloride, and the yellow oil which separates extracted with ether. The ethereal extract is washed and evaporated, the residual acetyl sorbitol dissolved in warm water, and the solution cooled in ice, when it yields crystals (m.pt. 98 to 99° C.). The procedure, which may be made more sensitive by the use of Jahr's method (ANALYST, 1930, **55**, 452), will detect additions of 2·5 per cent. of fruit wine, and is preferable to the production of benzylidine sorbitol (Werder, *id.*, 1929, **54**, 476). J. G.

Determination of Trigonelline in Raw and Roasted Coffees. F. E. Nottbohm and F. Mayer. (*Z. Unters. Lebensm.*, 1931, **61**, 202–210.)—The

method is a modification of that of Lendrich and Nottbohm (ANALYST, 1909, **34**, 214, 484). The sample (20 grms.) is extracted with chloroform, which removes more extraneous matter than carbon tetrachloride, the solvent removed completely, and the residue extracted 3 times, at hourly intervals, with 96 per cent. alcohol. The alcoholic liquor is mixed with excess of lead acetate solution (strength not stated), and the orange precipitate (yellow in dilute solutions) is filtered off on a large folded paper, washed with alcohol, and the lead removed from the filtrate by means of hydrogen sulphide. The resulting filtrate is evaporated to 50 c.c. with 5 c.c. of hydrochloric acid (sp. gr. 1·124) to destroy the sugars, the solution filtered from the oily deposit, and the filtrate evaporated with a little charcoal and again with water and a few drops of acid. The residue is warmed with water and more charcoal, filtered, washed with water, and the clear and colourless filtrate treated with 3 drops of hydrochloric acid and 11 c.c. of 0·1 *N* iodine solution. A cloudy brown precipitate results, which settles out in crystals after 10 minutes, when it is filtered on an asbestos Gooch filter, washed with a little cold water, dissolved in warm alcohol, and the diluted solution titrated with 0·1 *N* sodium thiosulphate solution (1 c.c. 0·1 *N* iodine solution ≡ 4·90 mgrms. trigonelline). Raw coffee gives steel-blue, spear-shaped crystals of the iodine compound, and roasted coffee an oily modification of the same compound (containing 1 mol. of trigonelline to 3 of iodine) crystallising slowly in leaflets. After the titration the solution may be shaken with silver oxide, filtered after some time, and the filtrate evaporated with excess of hydrochloric acid and a little charcoal. The residue is extracted with 96 per cent. alcohol (which leaves the silver and sodium chlorides) and filtered hot. The compounds $(C_7H_7NO_2)_4$, 3HCl, $3AuCl_3$ (large yellow needles, m.pt. 186° C.) and $C_7H_7NO_2$, HCl, $AuCl_3$ (m.pt. 198° C., 4-sided plates or prisms) are produced by precipitation directly with gold chloride and recrystallisation from hot dilute hydrochloric acid, and by precipitation of the hydrochloride with gold trichloride in the presence of acid and recrystallisation from a hot dilute acid solution of the gold salt, respectively. The compound $(C_7H_7NO_2)_2$,HCl,$AuCl_3$ m.pt. 248 to 249° C. (Gorter) is produced as yellow aggregates under conditions which are not easily defined. The sources, properties and formulae of the various gold chloride hydrochloric acid salts obtained by other workers are tabulated (*cf.* Lendrich and Mayer, ANALYST, 1931, 326). J. G.

New Triglyceride obtained on Oxidising Cocoa Butter. J. Bougault and G. Schuster. (*Compt. rend.*, 1931, **192**, 953–954.)—Oxidation of cocoa butter by Hilditch's method (ANALYST, 1929, **54**, 243) shows that nearly all the glycerides are unsaturated, and it was found possible to isolate a palmito-stearoazelate derived from the neutral palmitostearo-olein, owing to the solubility of the sodium salt in hot water. This glyceride is crystalline, melts at 58°–59° C., and is insoluble in water, slightly soluble in 95 per cent. alcohol, soluble in boiling alcohol, and in ether, acetone, and chloroform, but only slightly so in petroleum spirit. The molecular weight, saponification value and acid value accord with its being a palmito-stearoazelate. It was found possible, by saponification, to detach the

azelaic acid from the molecule, and a neutral palmito-stearin, functioning like a free alcohol and crystallising in mica-like leaves, melting at 34° C., was isolated. At least 36 per cent. of the palmito-stearic triglyceride is present in cocoa butter.

D. G. H.

Raw Tobacco. Nicotine Content and its Retention at Various Temperatures. C. Pyriki and H. Dittmar. (*Z. Unters. Lebensm.*, 1931, **61**, 210–217.)—The nicotine content of tobacco is dependent on the nature of the plant, the soil, the fertilisers used, climatic conditions, etc. In general, low nicotine contents are indicated by light colour and a thin leaf. The nicotine content is less in the flower than in the upper leaves (by 0·1 to 2 per cent.), and increases along the leaf from stem to tip according to the extent of exposure to sun. The stalks usually contain less than 0·5 per cent., and this accounts for the high nicotine contents of cigars from which these are removed. Pfyl and Schmitt's method (ANALYST, 1927, **52**, 728) gave the following percentages of nicotine in loose, fermented oriental tobaccos:—Grecian (19 samples), 0·75 to 3·41; Bulgarian (7), 0·63 to 3·17; Turkish (14), 0·90 to 2·17. Determinations of nicotine and of moisture content (by distillation with toluene, Gawrilow and Ewslina, *Biochem. Z.*, 1929, **208**, 79) made after 3 hours at 50° and 95° C. indicate that (particularly at 95° C.) the loss of nicotine is greatest from the moistest tobaccos. The moisture removed by the action of calcium chloride for 48 hours in a vacuum at the ordinary temperature is less than that expelled (in air) after 3 hours at 50° C.; after 3 hours at 120° C. 0·4 to 1 per cent. remains. The results also indicate that, after drying at temperatures above 95° C., low nicotine values (0·2 to 0·4 per cent.) are obtained, owing to volatilisation, but that below 95° C. the values agree to within 0·1 per cent. Other volatile basic substances were determined by steam-distillation of the tobacco and titration of 100 c.c. of the distillate with 0·1 *N* acid solution, and the results indicate that drying at a little above 50° C. produces a loss compared with desiccation over calcium chloride. In the case of a "nicotine free" tobacco examined by Petri (*Z. Unters. Lebensm.*, 1930, **60**, 123) lower results were obtained, and it is pointed out that the technique of Pfyl and Schmitt (*loc. cit.*) must be followed carefully, as a small titration error is multiplied in the final nicotine content. In particular, the dipicrate should be filtered on a small paper, alkali free from carbon dioxide should be used, and the titration carried out in a micro-burette; the blank on the reagents should be deducted.

J. G.

New Reaction for Capsaicin. K. v. Fodor. (*Z. Unters. Lebensm.*, 1931, **61**, 94–100.)—The sample is dried and powdered, and 2 grms. are shaken with 10 c.c. of dry acetone, allowed to settle for 3 hours (or centrifuged), 5 c.c. pipetted off, and 9 drops of hydrochloric acid added. This mixture is shaken gently with 0·1 grm. of ammonium vanadate. A blue colour results from 0·08 per cent. of capsaicin, varying through green to brown-green for 0·01 per cent., the limiting concentration for the test. The brown colour is probably due to oxidation of carotene and capsantin in the sample (*e.g.* capsicum), and hence provides another test, in which the brown colour (turning to light yellow) is produced by addition

of 3 drops of hydrochloric acid and 4 c.c. of 33 per cent. hydrogen peroxide to 5 c.c. of the acetone extract. For quantitative work it is preferable to prepare an extract in dry ether (shake for 45 minutes), and to add to 10 c.c. of this a 1 per cent. solution of vanadium oxytrichloride in carbon tetrachloride. This reagent, which is more stable and more sensitive than the ammonium vanadate reagent, should be added in drops till no further colour is developed, and excess avoided. The colour is stable for a week, and may be matched against a series of standards containing 0, 0·2, etc., to 0·8 c.c. of a 0·1 per cent. solution in acetone of capsaicin (extracted from paprika in alcohol). Capsaicin was absent from Spanish pepper, and 0·01 per cent. or less was found in refined Hungarian paprikas. The blue vanadyl capsaicin was prepared in bulk from the calculated quantities of pure capsaicin and vanadium oxytrichloride in carbon tetrachloride solution, washed with this solvent, and dried in a vacuum over phosphorus pentoxide at 80° C.; it was shown to have the composition $C_{18}H_{26}NO_3,VOCl_2$. J. G.

Purification of Common Salt [American]. T. B. Brighton and C. M. Dice. (*Ind. Eng. Chem.*, 1931, **23**, 336–339.)—The refined salt produced in the United States, particularly that from the Great Salt Lake, contains traces of substances which yield an objectionable odour when the salt is dissolved in warm water. The substances may be removed by washing the salt with brine and then heating it at a temperature not exceeding 225° C. W. P. S.

Determination of Choline and of Acetylcholine. L. Lematte, G. Boinot, E. Kahane, and M. Kahane. (*J. Pharm. Chim.*, 1931, **123**, 371–385.)—The assay of acetylcholine involves the quantitative determination of the acetylcholine present and also its index of decomposition. The determination may be by means of either phosphotungstic or silicotungstic acid; the method is the same for choline and acetylcholine. Since these substances are appreciably soluble in the mother liquors, the reactions are carried out in as concentrated a medium as possible, and washing is reduced to a minimum. If approximately 0·1 grm. of choline is present, 15 c.c. of a 10 per cent. solution of the tungstic acid are added, and after keeping for a few minutes on a boiling water-bath, the mixture is cooled and filtered. The precipitate is washed three times by decantation, dried for 1 hour at 105° C. and weighed, and the weight, multiplied by 0·131, gives the weight of choline. If the phosphotungstate is calcined for 15 minutes at red heat the dark green anhydride is formed; and the same procedure may be used with silicotungstic acid. Acetylcholine may also be weighed as the phosphotungstate, or its anhydride, or as the silicotungstate or its anhydride. Owing to solubility the results are consistently 2 to 3 per cent. below theory. The ashing of the acetylcholine phosphotungstate gives a residue of 86·38 per cent., and the ashing of choline phosphotungstate a residue of 89·74, whilst for a mixture the figure will be intermediate. A weighed portion of the dried precipitate is calcined, cooled and weighed again, and the ratio obtained compared with tables or curves giving the corresponding index of decomposition. D. G. H.

Biochemical.

Tin in the Animal Organism. G. Bertrand and V. Ciurea. (*Compt. rend.*, **1931**, **192**, 780–782.)—The proportions of tin present in the various organs of the body of the ox, the horse and the sheep were determined as follows:—The organs were removed from the carcase immediately after slaughtering and, after being cleaned, were heated for several days in a roomy quartz vessel with a mixture of sulphuric and nitric acids. After all the organic matter had been destroyed, the excess of acid was eliminated by evaporation in a platinum dish, the residue then treated with hydrochloric acid and the silica filtered off. After precipitation of the copper, lead, tin and platinum as sulphides by means of hydrogen sulphide, the precipitate was treated to convert the tin into stannic acid. For the various parts of the animal, the proportions of tin, in mgrms. per kilo of material, varied: for the ox, from 0·4 in the stomach to 9·48 in the skin; for the horse, from 0·65 in the stomach to 8·53 in the skin, and, for the sheep, from 0·68 in the large intestine to 6·20 in the skin. The tongue is even richer in tin, the figures for the muscle and mucus of this organ being 12·2 and 18·65 respectively for the ox, and 16·45 and 26·11 for the sheep. T. H. P.

Unsaponifiable Lipids of Lettuce. I. Carotene. H. S. Oleovich and H. A. Mattill. (*J. Biol. Chem.*, **1931**, **91**, 105–117.)—The recent work of various investigators leaves little doubt that carotene is the plant source of the vitamin *A* of animal tissue. The confirmation of this relationship tends to harmonise some discordant facts and presents further interesting problems, especially the chemical reactions of carotene and the mechanism of its transformation into vitamin *A*. A few observations upon the properties (physical, chemical and physiological) of carotene are now recorded, and further proof of the vitamin *A* activity of this substance obtained from another vegetable source, lettuce, is presented. Crystalline carotene (brilliant red pleochroic crystals) was obtained from the unsaponifiable lipids of lettuce. The 200 mgrms. isolated from 10 kilos. of dried lettuce, representing 140 kilos. of fresh leaves, were probably only a small fraction of the original content. Carotene crystallises in the hexagonal system; the external forms of the crystals are many and varied, depending on several factors, but, primarily, on the solvent; *e.g.* rhombohedrons were obtained from petroleum spirit, triangular plates from acetone, etc. It is shown that the fading of the colour of the crystals at high temperatures is not an oxidation. Carotene solutions may be bleached by heat, ultra-violet light, and by the presence of autoxidisable fats. The presence of hydroquinone delays for a variable length of time the bleaching of carotene solutions by any of the above mechanisms. Hydroquinone protects ethyl laurate solutions of carotene apparently indefinitely. Such solutions are satisfactory for physiological studies. In autoxidisable mixtures, carotene is an active prooxidant; this property is unique in a hydrocarbon. The physiological activity of carotene as vitamin *A* is confirmed; under widely differing conditions, the growth induced by feeding rats with carotene seems to be directly proportional to the

amount given; 0·005 mgrm. permits an increment of 3 to 5 grms. in the weight of rats deprived of vitamin *A*. Since carotene in the solid state is decolorised and rendered inert, physiologically, by heat in the absence of oxygen, and since in solution it undergoes this change even more rapidly under the influence of heat or ultra-violet radiation, it follows that the resultant achroocarotene is not a product of oxidation but rather of an intramolecular rearrangement, or possibly of polymerisation. Inasmuch as hydroquinone delays the transformation of carotene under these conditions, its function as an anti-oxidant must be extended to include the capacity to prevent the shift in electrons, if such it is, which attends the thermal or photoelectric change of the unstable to the colourless and more stable form of carotene. The pro-oxidant character of carotene, a capacity found in smaller degree in achroocarotene, shows that the former possesses greater molecular energy than the latter. The electronic significance of conjugate double bonds, of which carotene possesses eleven, is, as yet, obscure. Obviously the commonly measured stability of vitamin *A* (carotene) in various vegetable foods exposed to heat and light does not depend upon the carotene itself or on the presence of oxygen, primarily, but is conditioned by the presence of other substances. Some properties of achroocarotene are given. P. H. P.

Association of Vitamin *A* with Greenness in Plant Tissue. III. Vitamin *A* Content of Asparagus Grown under Light of Various Qualities. J. W. Crist and M. Dye. (*J. Biol. Chem.*, 1931, **91**, 127–134.)—Experimental evidence is presented which gives added support to the conception that in some manner or other the elaboration of vitamin *A* in the plant is connected with the development of the chlorophyll pigment. A brief summary is given of the conclusions reached by other workers on the subject. For the first time, a reasonably successful attempt has been made to establish this association on a quantitative basis. It was necessary to produce plants which would vary gradually in chlorophyll content from zero to a maximum quantity, and to determine their respective vitamin *A* efficiencies by the use of test animals. A preliminary experiment with varicoloured and uncoloured light filters gave every indication of the possibility of using such a method to secure asparagus tips of variable greenness whose vitamin *A* properties as food for test animals (rats) could be correlated with their degree of greenness. Accordingly, specially manufactured filters were obtained, and used in experiments with asparagus. From the results obtained it seems reasonable to conclude that, within the restrictions of the two variables expressed by some non-linear relationship, chlorophyll content is a limiting factor on vitamin *A* synthesis in the vegetative parts (in this case the stem tip) of the plant. This gives point to the contention of Schertz (*Science*, 1928, **68**, 48) that further progress in a knowledge of vitamin *A* may depend much upon the industry and the success of the biochemist and the plant physiologists in their efforts to solve the mysteries of chlorophyll and its functions. P. H. P.

Chemistry of Vitamin B_2. B. C. Guha. (*Nature*, 1931, **127**, 594–595.)—Cold aqueous extract of commercial liver extract is rich in vitamin B_2, being

effective in producing good growth in young rats on a B_2-deficient diet, in a daily dose representing 40–60 mgrms. of the original liver extract. The vitamin is not precipitated by picric acid, benzoyl chloride, flavianic acid, or litharge, and it is not precipitated or destroyed by nitrous acid. Neutral lead acetate partially precipitates it at *p*H 4·6 or 7·0, and silver nitrate precipitates the bulk of the active material, but baryta does not precipitate it in either aqueous or 50 per cent. alcohol solution. Norit adsorbs the factor at the natural *p*H (4·6) of the aqueous liver extract, but this cannot be elutriated by acid, alkaline or neutral water-alcohol mixtures or by dilute saponin solution. Phosphotungstic acid gives an inactive precipitate and a slightly active filtrate, a combination of these two being equally unsatisfactory. Esterification with ethyl alcohol leaves the bulk of the activity in the non-esterified portion, the ester being almost inactive. Trypsin is without effect on the vitamin, which is stable to sulphur dioxide, hydrogen peroxide, and ozone. If the vitamin is a single chemical entity, it is probably not a base, an acid, or a peptide, but a neutral substance, this conclusion being supported by the results of experiments on the electro-dialysis of vitamin B_2. This vitamin appears to be fairly readily adsorbed by neutral precipitates, so that its partial precipitation by lead acetate and by silver nitrate is probably due to adsorption on the precipitates formed. Liver extract is potent in both vitamin B_2 and the factor specific for pernicious anaemia, but evidence, based partly on the methods of their fractionation, indicates these to be different.

Commercial liver concentrate and commercial yeast extract (marmite) are both fairly stable to autoclaving at 124° C. at *p*H 9, whereas aqueous extracts made from brewer's yeast, fresh ox-liver, and ox-muscle are markedly unstable under these conditions. The stability appears to be connected with the presence of certain protective materials in a given fraction. T. H. P.

Bacteriological.

Thermophilic Bacteria in Milk. M. I. Christian. (*Nature*, 1931, **127**, 558.)—The sudden and violent fluctuations in the numbers of thermophilic bacteria in milk during pasteurisation at 62·8° C. led Mudge and Thorwaldson (*Milk Dealer*, Dec., 1930, 57) to suggest that the organisms are present as dormant spores which remain ungerminated, unless the milk is subjected to the action of certain physical and chemical stimuli such as heat, cold, or the action of alkalis. Support for this view is furnished by results obtained by the author with a spore-forming organism isolated from commercial sterilised milk. If milk is heated after inoculation with the spores, germination always occurs; otherwise, germination is greatly reduced and fails in the second generation. The original spores are gradually lost by a process of dilution during subsequent cultivation, and a stable vegetative form of the organism is obtained. If a small quantity of a living vegetative culture is added to a culture of heated spores capable of germination, a number of these immediately lose their germinative power and all lose it after 24 hours. If, however, the vegetative culture is killed prior to its addition to the spores, these undergo

germination, followed by normal spore formation. Apparently the stable vegetative form, which has been found to dissociate from the sporing form, bears an inhibiting factor, which is destroyed by heat. The property of lying dormant, which is possessed by the spores of thermophilic bacteria in milk, and which is lost on heating, is possibly due to the presence of a similar inhibitory factor. T. H. P.

Toxicological and Forensic.

Poisoning by Methyl Alcohol. A. Sartori. (*Chem. Ztg.*, 1931, **55**, 259.)—Dissection, 6 days after death, of the body of a man who died during the early years of the War after drinking Russian spirit having indicated no definite cause of death, a mixture of portions of the various internal organs and their contents, with the blood and urine, was mixed with 20 per cent. of salt and distilled in a current of steam. A part of the distillate of about 500 c.c. (from 970 grms. of material) gave a negative result when tested for formaldehyde by boiling with milk and a hydrochloride acid solution of ferric chloride. The remainder of the distillate was filtered, and the filtrate neutralised accurately, mixed with 20 per cent. of salt and distilled to yield 170 c.c. of distillate. A few c.c. of this liquid were acidified with dilute sulphuric acid, heated with a small amount of potassium permanganate to 50° C., and filtered. When boiled with milk and a mixture of ferric chloride, the clear filtrate assumed an intense violet coloration. Another portion of the second distillate gave a distinct violet colour when mixed, with cooling, with concentrated sulphuric acid and a solution of morphine sulphate in the same acid. These reactions indicate the presence of formaldehyde in the oxidised distillate, and hence that of methyl alcohol in the body organs examined.

To test these organs for formic acid, the residual liquid remaining after the second distillation was strongly acidified with phosphoric acid and distilled. The distillate was acidified with sulphuric acid, digested for some hours with magnesium turnings, and filtered. When boiled with milk, hydrochloric acid and ferric chloride, the liquid then gave no violet colour, the absence of formic acid from the organs being thus indicated.

Similar results were recently obtained in the case of a soldier found dead in a train from Russia. T. H. P.

Colorimetric Determination of Traces of Cadmium in Organic Matter. L. T. Fairhall and L. Prodan. (*J. Amer. Chem. Soc.*, 1931, **53**, 1321–1323.)—The organic matter (100 grms.) is destroyed by digestion with nitric acid; after addition of sulphuric acid (10 c.c.) the oxidation is continued until white fumes are freely given off by the colourless solution. The liquid (75 c.c.) is treated with 0·5 mgrm. of copper as sulphate, 2 grms. of sodium citrate, ammonia to a hydrogen ion concentration of 10^{-3} (thymol blue and bromchlor-phenol blue), hydrogen sulphide for 5 to 10 minutes, and a drop of 5 per cent. aluminium chloride solution. After standing for 6 to 12 hours, the precipitate is collected, dissolved in nitric and hydrochloric acids, and the solution evaporated to dryness. The sulphide precipitation is repeated twice more, the sodium citrate being omitted the last time,

CH adjusted to 10^{-2} by means of dilute potassium hydroxide solution, and the sulphide dissolved as before. The final chloride solution is evaporated to dryness, the residue dissolved in water, made up to bulk, and an aliquot part transferred to a Nessler tube. To this tube are added 5 drops of 10 per cent. potassium cyanide solution, distilled water, and 5 c.c. of hydrogen sulphide water. The colours of the solutions are matched against those of a standard cadmium solution in the light of a quartz mercury vapour lamp. The ultra-violet rays produce an intensification of the yellow colour of the sulphide, 0·01 mgrm. of cadmium in 50 c.c. producing a perceptible difference in tint. An accuracy of 4 per cent. is claimed for contents of 0·4 to 1 mgrm. of cadmium in 100 grms. of organic material. The reagents used should be free from lead. Dark or turbid solutions indicate that iron has not been completely removed. The standard tubes should be prepared at the same time as those containing the solution under investigation, and should not be allowed to stand overnight. W. R. S.

Organic Analysis.

Effect of Light on the Determination of Ethylene. J. L. Oberseider and J. H. Boyd. (*Ind. Eng. Chem., Anal. Edition*, 1931, **3**, 123.)—Ethylene can be separated sharply from saturated paraffin hydrocarbons by absorption in one-third saturated bromine water in a Williams pipette, provided that the apparatus is shielded from direct sunlight and the glass is painted black. In direct sunlight saturated paraffin hydrocarbons are slowly absorbed by the reagent. S. G. C.

Use of Buffers in the Determination of Colours (Dyes) by means of Titanium Trichloride II. O. L. Evenson and R. H. Nagel. (*Ind. Eng. Chem., Anal. Edition*, 1931, **3**, 167–169.)—A method is given for the evaluation of the colouring matter in water-soluble coal-tar food colours which are on the permitted list of the United States Department of Agriculture. It is based on Knecht and Hibbert's method, and is as follows: In the case of triphenylmethane dyes, the solution (200 c.c.) of 30 grms. of the buffer salt required, as noted below, is heated to boiling, and cooled to 85° C. under carbon dioxide; 1 grm. of the dye dissolved in 100 c.c. of water is added, and the solution is titrated with standardised titanous chloride at 60° to 70° C. For other dyes, 10 to 20 grms. of the buffer salt are used, and the volume of the solution should be approximately 100 c.c. at the start of the titration, which is carried out with the solution hot under carbon dioxide, and stirred mechanically. A table is given in the paper showing the most suitable buffering agents for particular dyes. Sodium bitartrate, Rochelle salt, or sodium tartrate is recommended for Orange I (150), Ponceau SX, Tartrazine (640), Naphthol Yellow (10), Indigotine (1180), Brilliant Blue FCF, Fast Green FCF, Light Green SF Yellowish (670), Guinea Green B; and sodium citrate for Amaranth (184), Ponceau 3R (80), Sunset Yellow FCF. The numbers in parentheses following the name of each dye are those given in the 1924 edition of the Colour Index. S. G. C.

New Higher Alcohols produced during the Hydrogenation of Fish Oils. S. Ueno and R. Yamasaki. (*J. Soc. Chem. Ind., Japan*, 1931, **34**, 35B.)—One kilo. of unsaponifiable matter from a fish oil was washed with methyl alcohol in order to separate the oxy-compounds from the hydrocarbons. The portion soluble in methyl alcohol was freed from the minute amounts of malodorous aldehydes by means of sodium bisulphite, washed with water, and dried over anhydrous sodium sulphate, yielding 20 grms. of higher alcohols. These crude oils were distilled at 15 mm. pressure and collected in six fractions ranging from 90–100° C. to 150–163° C. in b.pt. From the ultimate analysis, molecular weight and iodine value, the empirical formula for the first fraction is given as $C_9H_{18}O$; and for the last fraction $C_{14}H_{26}O$. The m.pts. of the alcohol and of the acid produced by oxidation with permanganate are too low to correspond with the primary alcohol, and it is assumed that fraction 6 is, therefore, an aliphatic iso-primary alcohol.

R. F. I.

Quantitative Determination of Mixtures of Isomeric Unsaturated Compounds. A Review of the Iodimetric Methods and a New Bromimetric Method. R. P. Linstead and J. T. W. Mann. (*J. Chem. Soc.*, 1931, 723–725.)—The original iodimetric method for acids (*J. Chem. Soc.*, 1927, 2565) has been found in practice to be very successful for the monobasic series, but the high reactivity of the $\beta\gamma$ acids towards iodine diminishes with the entry of negative groups. All the $\alpha\beta$ acids so far examined have iodine additions of 0 to 1 per cent. by the standard method, and this provides a valuable test of their purity. The estimation of mixtures of itaconic and mesaconic acids presents difficulties, since the addition of iodine under the standard conditions was negligible, but addition of bromine in most solvents was too rapid for convenient measurement. The method adopted is to standardise a 0·05 N solution of bromine in concentrated aqueous potassium bromide (400 grms. to 1 litre), and to run 25 c.c. quantities of this solution from the burette into 10 c.c. of water in stoppered bottles. After keeping for 10 minutes in the dark, the solutions are treated with 10 c.c. of 10 per cent. aqueous potassium iodide, and the liberated iodine titrated with 0·05 N thiosulphate, after which 10 c.c. of exactly M/15 solutions of the pure acids or their mixtures are pipetted into stoppered bottles and treated with an exactly equivalent amount of the bromine solution, calculated from J (amount required in c.c.) $= 100/3t_{25}$ N, where t_{25} is the thiosulphate titration of 25 c.c. of the bromine, and N is the normality of the thiosulphate. The back titration is carried out as for the blank. The amount of addition to the pure acids varied with temperature, from 37·3 for mesaconic and 67·3 for itaconic at 16·8° C., to 45·9 and 75·7 at 23·2° C., but the temperature coefficient is the same in each reaction, so that a reference curve was constructed and enabled a simple temperature correction to be made. For mixtures of mesaconic and itaconic acids containing 100 per cent. mesaconic acid (at 16·8° C.) the percentage addition is 37·3; 80 per cent., 43·3; 60, 49·5; 40, 55·9; 20, 62·2; and 0, 67·3. The figures obtained for the compositions of mixtures were found to be accurate to ± 1 per cent., and agreed with those determined by actual isolation of the itaconic acid.

D. G. H.

Preparation and Properties of Highly Purified Oleic Acid. J. H. Skellon. (*J. Soc. Chem. Ind.*, 1931, **50**, 131–134T.)—Two modified processes of existing methods for the preparation of pure oleic acid are given. The liquid acids are separated from the crude acids of olive oil by a modification of Twitchell's lead salt and alcohol process by adding a solution of 200 grms. of potassium hydroxide in 500 c.c. of water to a mixture of 1 kilo. of olive oil with 2 litres of absolute alcohol, and, after refluxing for 3 hours, removing the excess of alcohol by distillation under reduced pressure, boiling the syrup with 10 per cent. hydrochloric acid, and pouring the free acids into water. Mineral acid is removed by washing, and the mixture is dissolved in 4 times its weight of ethyl alcohol and heated to boiling, after which sufficient lead acetate (dissolved in an equal volume of boiling alcohol) to combine with 24 per cent. of the total acids is added, and after standing for 12 hours the lead salts are filtered off, redissolved in hot alcohol with a few drops of acetic acid, boiled, cooled and filtered. Alcohol is removed from the filtrates, and the acids, liberated from the syrup by 10 per cent. hydrochloric acid, are extracted with ether. I. The liquid acids, amounting to 60 per cent. of the crude acids, are dissolved in 12 times their weight of a mixture in equal volumes of dry benzene and absolute alcohol, and sufficient barium hydroxide is added to the boiling solution for neutralisation to phenolphthalein, and a small amount of hot water. After rapid cooling, the oleate is collected, dried, recrystallised from 3 times its weight of moist benzene and absolute alcohol, the purified oleate suspended in warm water and decomposed by repeated shaking with 10 per cent. hydrochloric acid. On cooling, the upper layer of oleic acid is extracted with ether, dried, the solvent removed, and the oleic acid finally dried *in vacuo*, after which it is converted into the methyl ester. This is distilled under reduced pressure, and the main fraction (97 per cent. of the esters) saponified, and the acids recovered. The iodine value of the final, almost colourless, product is 90·5–91. II. The liquid acids are dissolved as in I; lithium hydroxide (about 20 per cent. of the weight of the acids) is dissolved in an equal volume of boiling water, and this is gradually added to the boiling solution of the acids under a reflux condenser, until they are neutral to phenolphthalein. On cooling, the oleate is filtered off, washed, dried, and recrystallised twice from four times its weight of alcohol, decomposed by 10 per cent. hydrochloric acid, and the recovered oleic acid further purified by conversion into, and distillation of the methyl ester. The iodine value of the final product is 90 to 90·8. The refractive index, n_D^{20}, for highly purified oleic acid is taken as 1·4610, falling slightly after 2 years; the setting point as 11·8° to 12·2° C., falling about 1° C. after a year, and the m.pt. as 13°–14° C. D. G. H.

Rosinduline as Oxidation-Reduction Indicator. L. Michaelis. (*J. Biol. Chem.*, 1931, **91**, 369–372.)—Rosinduline is recommended as an indicator for oxidation-reduction potential in a very negative potential range. Its normal potential at 30° C., referred to the normal hydrogen electrode, is

*p*H	5	6	7	8	9	10	11
Normal potential, volts	−0·161	−0·221	−0·281	−0·340	−0·395	−0·438	−0·480

The dye, Rosinduline 2G, is the sodium salt of a monosulphonic acid of a compound called Rosindon. The dye has an intense scarlet red colour; the leuco-dye in higher concentrations is slightly yellow-brown, but colourless in the lower concentrations in which the dye may be used as an indicator. The dye is readily soluble and stable, both in the oxidised and reduced state, even in very strongly alkaline solution, and so differs, advantageously it is thought, from all dyes of a comparable negative potential range; its potential range is even more negative than is that of any others which are recommended. The potentials are perfectly reproducible and steady, even at very high *p*H, so that this dye may be used for class experiments for reversible oxidation-reduction titration curves. P. H. P.

Inorganic Analysis.

Detection of Cadmium. J. S. Pierce and W. T. Forsee. (*Ind. Eng. Chem., Anal. Edition*, 1931, **3**, 188.)—Boiling the solution with nickel powder, as described below, is advocated for removing copper, lead, mercuric mercury, and bismuth prior to testing qualitatively for cadmium by hydrogen sulphide. To the original solution, neutral or slightly acid, and containing an excess of sulphate ions but no acetate, is added 1 to 5 grms. of fine nickel powder (100 mesh or finer), and the whole boiled for from 3 to 5 minutes. The liquid is filtered. A portion of the filtrate is brought to an acidity of 2 *N* in acetic acid, and hydrogen sulphide passed into it for 15 seconds, when the appearance of a yellow precipitate indicates the presence of cadmium. If the precipitate is dark coloured, indicating incomplete removal of the other metals, the remaining portion of the solution is treated with more nickel powder. The presence of the sulphate is required to precipitate the lead as sulphate, because lead is not readily removed by nickel powder. Cadmium is partially precipitated by the nickel powder, but enough remains in the solution to respond to the test with hydrogen sulphide. S. G. C.

Structure of Ferric Thiocyanate. H. I. Schlesinger and H. B. Van Valkenburgh. (*J. Amer. Chem. Soc.*, 1931, **53**, 1212–1216.)—The red colour produced by the addition of thiocyanate to ferric salt is usually ascribed to ferric thiocyanate. The compound is soluble in ether, which extracts it from water; the ethereal solution does not contain alkali, which proves that the coloured substance is not $M'_3[Fe(CNS)_6]$. When the aqueous red solution is electrolysed, the red colour migrates toward the anode, while ferric ion is found at the cathode; the complex nature of the compound is further borne out by the molecular weight, which, in non-aqueous solution, is practically twice that of $Fe(CNS)_3$. The authors conclude that the formula $Fe[Fe(CNS)_6]$ should be ascribed to the ether-soluble substance. W. R. S.

Determination of Thiosulphate by Means of Ceric Sulphate. N. H. Furman and J. H. Wallace, Jr. (*J. Amer. Chem. Soc.*, 1931, **53**, 1283–1288.)—The thiosulphate solution (0·5 to 0·7 grm. of salt) is treated with 0·3 to 0·4 grm. of potassium iodide and starch solution, diluted to 250 c.c., and titrated with 0·1 *N* ceric sulphate solution. The results are stoichiometric. W. R. S.

Separation of Calcium and Magnesium by the Molybdate Method. R. C. Wiley. (*Ind. Eng. Chem., Anal. Edition*, 1931, **3**, 127–129.)—To the boiling, concentrated, neutral or slightly ammoniacal solution containing the calcium and magnesium is added slightly ammoniacal ammonium molybdate solution (about 0·4 *N*), at the rate of about one drop per second, until an excess is present. The boiling is continued until the precipitate of calcium molybdate has settled (about 10 minutes' boiling is required), the solution is kept until cold (about 45 minutes); the precipitate is filtered off on a weighed Gooch crucible, with the aid of gentle suction, and washed 10 times with 10 c.c. of hot water. The crucible is heated at 130° C. for 30 minutes and then ignited before weighing. The factor for converting calcium molybdate into calcium is 0·2002. The magnesium in the filtrate is determined as magnesium pyrophosphate with, however, only a single precipitation of the magnesium ammonium phosphate. Good results were obtained in test experiments with mixed solutions of calcium chloride and magnesium nitrate, with and without the addition of several grms. of ammonium chloride. A large excess of ammonium molybdate should be avoided in the precipitation; before the filtration, a drop of the liquid should be tested with a saturated solution of pyrogallol in chloroform, when a brown colour indicates an excess of molybdate. S. G. C.

Determination of Lanthanum (New Colorimetric Method). I. M. Kolthoff and R. Elmquist. (*J. Amer. Chem. Soc.*, 1931, **53**, 1217–1225, 1225–1232, 1232–1236.)—The determination of lanthanum after precipitation as oxalate and as hydroxide was studied by precision methods. The solubility of the two compounds was investigated with the help of a new colorimetric method based on the bright violet coloration given by sodium alizarinate in solutions containing as little as 0·1 mgrm. of lanthanum per litre in acetate solution. The filtrates from the oxalate precipitates were evaporated with excess of sulphuric acid to complete dryness, and the residue taken up in one c.c. of a solution of ammonium acetate and acetic acid (both 2 *N*) and 10 c.c. of water, and the reagent (0·4 c.c. of 0·1 per cent. sodium alizarinate solution) added; a solution of known lanthanum content was used as the standard. The coloured compound flocculates after some hours. The method can be used for concentrations between 0·1 and 2 mgrms. per litre. The solubility of the oxalate was found to be 2·08 mgrms. of anhydrous salt, that of the hydroxide 0·7 mgrm. of La_2O_3, per litre at 25° C.

Oxalate precipitation.—The determination can be made by the volumetric method, either the excess oxalic acid in the filtrate, or the solution of the washed precipitate in warm dilute sulphuric acid being titrated. It is, however, indispensable to wash the precipitate very thoroughly with water so that the adsorbed oxalic acid may be completely removed. A large excess of precipitant is required for quantitative precipitation, *e.g.* 50 c.c. of *N* oxalic acid for 0·15 grm. of La_2O_3. After standing overnight the precipitate is collected and washed with cold water until methyl orange gives with the washings the same colour as it does with the wash

water, then with about 175 c.c. more water (about 275 c.c. in all). The washed precipitate is dissolved in warm dilute sulphuric acid and the solution titrated with permanganate. Accuracy within 0·2 per cent. is claimed. Neither for the volumetric nor for the gravimetric method should alkali oxalate be used, as there is considerable co-precipitation, especially of the potassium salt. The authors' investigations prove that this is due to double-salt formation. For gravimetric work, the oxalic acid solution in excess (*vide supra*) is added, with constant stirring. The precipitate is filtered off the next day and ignited; drying yields no compound of constant weight at any stage of the operation. The ignition must be continued for several hours at 850° C. in an electric muffle, *i.e.* till constant weight is attained (*cf.* ANALYST, 1930, **55**, 650). The results of test experiments were consistently high by 0·15 per cent.; the authors express preference for the volumetric method.

Hydroxide precipitation.—Lanthanum can be determined volumetrically by precipitation as hydroxide and measurement of the amount of standard acid required to dissolve the precipitate. If the alkali—sodium or ammonium hydroxide—is added to the lanthanum solution, the results will be low, as the precipitate contains basic chloride. If, however, the lanthanum solution is slowly added to a fivefold excess of alkali, the results closely agree with the calculated values. The precipitate is collected the next day, washed with 50 per cent. alcohol till free from ammonia (phenol red test), dissolved in excess of standard acid, and the excess determined with standard alkali and methyl orange. Alternatively, a gravimetric process may be used: the hydroxide precipitate, produced and collected as described, is washed with dilute ammonia, ignited to constant weight as before, and weighed in a closed weighing bottle. This precaution must always be adopted when lanthana is weighed, as it attracts moisture and carbon dioxide. The oxide obtained by ignition of the oxalate or hydroxide to constant weight at 850° C., when subjected to prolonged ignition at a white heat, shows marked increase in weight (up to about 20 per cent.). The authors ascribe this to the formation of higher oxides.

W. R. S.

Microchemical.

A System for the Microchemical Identification of Alkaloids. J. F. H. Amelink. (*Pharm. Weekbl.*, 1931, **68**, 159–185.)—The system for the identification of 78 of the alkaloids utilises the formation of crystalline reaction products or precipitates, with one or more of 8 reagents, the reagents being chosen so that the reaction of each alkaloid with the 8 reagents is unlike the others. A complete table of the results of the action of each of the 8 reagents on the 78 alkaloids is given, together with 203 drawings of the microscopic appearance of the various crystalline products formed.

The reagents used are:—(1) A 10 per cent. platinic chloride solution, both in neutral solution and in dilute hydrochloric acid solution (0·5 *N*). Sometimes sodium iodide is added with a little platinic chloride solution, when the iodoplatinate is formed, which is usually less soluble than the chloroplatinate. (2) A 5 per

cent. gold chloride solution, used both in neutral and in dilute hydrochloric acid solution (0·5 *N*). Gold bromide is also sometimes used, as the bromo-aurates are usually less soluble than the chlorides. (3) Mercuric chloride, either in neutral or in dilute hydrochloric acid solution (0·5 *N*), when double salts may be formed. (4) Potassium ferrocyanide in dilute hydrochloric acid solution (0·5 *N*). This reagent is used warm. (5) Potassium ferricyanide in dilute hydrochloric acid solution. A large excess is always used. (6) Dragendorff's reagent, which is prepared by heating 18 c.c. of water, 3 c.c. of 4 *N* hydrochloric acid and 7 grms. of potassium iodide, and slowly adding 1·5 grm. of basic bismuth nitrate to the boiling solution. On cooling, 1·5 grm. of iodine is added, and the solution diluted with an equal volume of water, in which condition the solution will keep. The reagent is used in two different strengths, one concentrated, diluted with an equal volume of water, and the other very dilute. (7) A 50 per cent. solution of potassium hydroxide. (8) Picrolonic acid (dinitro-methyl-phenyl-pyrazolon), used as a fine crystalline solid; it is added to the neutral solution of the alkaloid. J. W. B.

Microchemical Identification of Harmine and Harmaline. J. F. H. Amelink. (*Pharm. Weekbl.*, 1931, **68**, 221–229.)—The properties and reactions of Merck's preparations, "Harmine" and "Harmaline," are described. "Harmine" is identical with banisterine hydrochloride, $C_{13}H_{12}ON_2HCl.2H_2O$. The alkaloid melts at 263° C. without decomposing. Harmine (the hydrochloric acid salt) is a white crystalline powder; the solubility in water at 20° C. is 1:40 and the solution is slightly fluorescent. With sulphuric acid the solution is yellow with a green fluorescence. With Fröhde's reagent it gives a yellow-brown colour, turning green on heating; with Erdmann's reagent a green colour, turning blue-green on warming; with Marquis' reagent a red-brown colour, turning violet on warming; and with Wasicky's reagent a yellow-brown colour which disappears on heating, and, on standing, becomes red-brown again, then red, and, finally, purple. When 0·05 mgrm. of harmine per grm. weight of the animal to be tested is injected intravenously, the first result is violent trembling, followed by cramp and death. Harmaline gives the same result when 0·005 mgrm. per grm. body weight is administered. The alkaloids can thus be identified physiologically. Chemical identification is carried out, using the 8 reagents described in the previous paper (abstract above). Harmaline, which is dihydro-harmine, melts (without decomposition) at 238° C. Merck's preparation is the hydrochloric acid salt. It is a yellow crystalline powder, yielding a yellow aqueous solution with a blue-green fluorescence, visible at a dilution of 1:1,000,000; this is a specific reaction for harmaline. When the alkaloid is heated with acetaldehyde on the water-bath a blood-red fleck is formed, soluble in chloroform or 90 per cent. alcohol, but not in ether; this can also be used as a test for acetaldehyde. Harmaline gives with concentrated sulphuric acid an intense yellow colour, turning light yellow on heating; with Fröhde's reagent, a brown-yellow colour, turning yellow-green on heating; with Erdmann's reagent, a brown-yellow colour, turning orange-yellow on heating; with Marquis' reagent, a red-brown colour becoming darker, and changing to green

on warming; with Wasicky's reagent, a yellow-brown colour, turning red-brown on heating. The reactions with the 8 reagents (see previous abstract) are carried out with either a neutral 1 per cent. solution or a solution in 0·5 *N* hydrochloric acid. Details of the results of the tests are given in the original, together with photomicrographs of the crystalline reaction products. J. W. B.

Physical Methods, Apparatus, etc.

Use of Tungsten Arc Lamps for Photomicrography. E. E. Jelley. (*Nature*, **1931**, **127**, 200–201.)—Owing to their uniform intrinsic brilliancy and compactness, these lamps are extensively used as illuminants for photomicrography, but it is not generally recognised that the light which leaves the metal surfaces at almost tangential angles is so strongly plane-polarised that it is difficult to obtain uniform illumination when crystals are being photographed by plane-polarised light. This lack of uniformity, which is not observed with either a carbon arc lamp or the sun, may give rise to appreciable errors if a tungsten arc lamp is used for spectrophotometry without the interposition of ground glass to form a secondary source. T. H. P.

Errata.—P. 334, l. 4, *for* "ClO_3" *read* "$KClO_3$."
l. 5 from bottom, *for* "determined from the data . . ." *read* "determined. From the data . . ."

Reviews.

BELL'S SALE OF FOOD AND DRUGS ACTS. By R. A. ROBINSON. Eighth edition. Pp. xxvi, 299, and (Index) 34. London: Butterworth & Co. (Publishers), Ltd., Bell Yard, Temple Bar; Shaw & Sons, Ltd., 7–9, Fetter Lane, E.C. 1931. Price 15s. net.

The first edition of "Bell" was noticed in THE ANALYST of 1886, and the reviewer has proved himself to be an excellent prophet, for he wrote (p. 139): "It will doubtless have a large sale among all officers engaged in carrying out the provisions of the Acts." To those who have been familiar with this work in its later editions, this latest, the eighth, will appear almost as a stranger. It is, however, a handsome stranger, and becomes more friendly on extended acquaintance, in spite of the fact that it does not fit well into a bookcase along with its forerunners.

The present edition is edited by Mr. R. A. Robinson, who is well known as the chief officer of the Public Control Department of the Middlesex County Council, and also as the author of the notes on adulteration which were a useful feature of previous editions. Mr. Robinson's reputation is such that the book is approached with pleasurable anticipation, an anticipation which is thoroughly realised by its subsequent perusal. In a standard work, such as this, praise seems no longer to be necessary, and it follows that any adverse criticism will become more prominent than its importance warrants. The points mentioned below, therefore, may be taken merely as suggestions for consideration by the editor for future editions.

In an introduction which is a valuable new feature of this edition, as compared with the last, although there was one in the sixth edition, the duties of Food and Drugs Authorities are well set out and carefully distinguished from those of Sanitary Authorities. The Milk and Dairies (Consolidation) Act, 1925, which was in the appendix of the last edition, now follows on immediately, and the Merchandise Marks Act of 1926 and the Sale of Food (Weights and Measures) Act, 1926, are also included.

The real meaning of the regulations referring to the labelling of condensed skimmed milk is clearly set out. There seems to be an impression in certain quarters that this refers to ordinary skimmed milk. The case of *Lamy* v. *Watson* under the Merchandise Marks Act is not mentioned. This is the case which decided that no trader or body of traders could set up a standard for any food, unless it was the ordinary basis of contract between the buyer and seller. The reviewer has not been able to trace an official report of this case.

Section 27 (5) of the Food and Drugs Act enacts that the name of the prosecutor shall appear on the summons. It has been held by several benches of magistrates that the mere appearance of the name is not sufficient, but that it must be preceded by some such words as "The name of the prosecutor is . . ." It would be an advantage if this point were stressed.

As was recently pointed out in THE ANALYST (1930, **55**, 40), the note on *Bakewell* v. *Davis* is not correct. In this case, as the Public Analyst had supervised the essential parts of the analysis, it was held that this was equivalent to the Analyst doing the analysis himself. This case, therefore, gives no support to the suggestion that a Public Analyst may rely entirely on the work of his assistants.

The suggested form for milk certificates, set out on page 182, does not appear to be the best; it might be advisable to give alternative suggestions.

The bibliography of official publications, given in Appendix I, is useful, and might be extended by the addition of the publications of the Food Investigation Board and the Empire Marketing Board, many of which are likely to be of interest to Public Analysts and Sanitary Officers.

The chemical notes might be extended with advantage. No mention is made of the adulteration of almonds with arachis nuts, or of arrowroot and cornflour with sweet potato, the sale of butter and margarine mixtures, the judgment of the

Staffordshire Stipendiary with regard to rice flour in shredded suet, etc. The remarks about glucose in the article on golden syrup (p. 292) are likely to be misunderstood by non-chemical readers and should be revised.

As already stated, these are only minor suggestions for the next edition. The book has for years been indispensable to all those whose work brings them into touch with the Food and Drugs Acts, and this latest edition will ably carry on the tradition. It is well bound and legibly printed on good paper. Typographical errors appear to be very few.

G. D. ELSDON.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. LIEFERUNG No. 308. By Prof. Dr. EMIL ABDERHALDEN. Section A, Part II, Subsection 5, containing: Die Methoden der Ligninforschung, by Walter Fuchs; and Die Terpene, by Konrad Bournot. Pp. 877–1068. Berlin and Vienna: Urban und Schwarzenberg. Price 10 gold marks.

Abderhalden's Handbuch covers anything from Chemistry to Psychology. It is obviously a very useful, but, at the same time, a highly expensive work. It is very hard to see how any one specialist could be interested in all that is to appear and already has appeared in it, and most subscribers find themselves by now in the position of the reviewer, who had to cancel his subscription. The age of the encyclopaedists is past and gone. Lieferung 308 may serve as a good illustration of the reviewer's contentions. It is written for highly specialised work, and it is most unlikely that one and the same research chemist could be interested in two such different subjects as lignin and the terpenes. Similar mixtures are to be found in most of the other 307 parts which have appeared.

The section on lignin is particularly well written, and Dr. Fuchs has shown great discrimination in selecting his material. Lignin has been the object of many recent investigators, and Freudenberg's results are well known to disagree with those of other workers. Fuchs, therefore, has done a good service to chemistry by balancing, in a critical manner, the divergencies of opinion between Freudenberg and his opponents. His description of the methods of investigating lignin is, therefore, a very valuable contribution to the literature.

Section 2, which deals with the terpenes, shows very little originality. It is nothing more than a compilation of facts which are accessible in most textbooks of Organic Chemistry. No specialist would find it a help in his work, and the general chemist is scarcely interested enough to require 130 pages on the subject.

M. NIERENSTEIN.

TEXTBOOK OF QUANTITATIVE ANALYSIS. By W. T. HALL. Pp. vii+279, with 42 illustrations. London: Chapman & Hall. 1930. Price 12s. 6d. net.

Many chemists throughout the world are familiar with "Treadwell and Hall" as one of the most reliable works on general analytical methods, although it was

originally produced as a students' textbook. The suggestion had been made that it should again be modified to suit its original purpose, but, instead, an entirely new volume, under the above title, has been produced by the translator of the original work.

The volume is based on a course in analytical chemistry provided at the Massachusetts Institute of Technology; and, although the methods described are almost identical with those in the older work, it is obvious that this textbook is far from being a mere abridgment of its predecessor. The subject-matter includes the usual requirements of the student, such as the use and calibration of apparatus, calculations, volumetric neutralisation, oxidation and precipitation methods. These are followed by the principles of gravimetric analysis and numerous applications of them, electrolytic analysis and a few more specialised methods, such as the estimation of carbon and nickel in steel and of tungsten and titanium. The last chapter is devoted to potentiometric titrations using hydrogen and quinhydrone electrodes, and the volume ends with a number of tables providing data required in calculations, etc. At the end of each chapter a series of "Home Problems" is provided, and any student capable of working these correctly without assistance would be thoroughly conversant with the mathematics of the subject.

The methods given have been well selected and are described with such detail that the highest degree of accuracy possible may be attained, an essential which is emphasised throughout the volume. In this connection a discussion of errors in the precipitation of barium sulphate by various reagents, given on pages 170–173, is invaluable to the student, although such a feature is but rarely met with in a textbook. It is, perhaps, a matter of opinion as to how much theory should be included in an essentially practical work, but an account of the theory of electrolysis extending to nearly 12 pages and a brief exposition of the theory of logarithms appear rather out of place.

The book is a model of careful production and is almost free from errors, only one, the trifling omission of an "o" giving "Ido Starch Reaction," being detected, while the index is complete and accurate and the illustrations clear, although there is no obvious reason why that on p. 40 should be reproduced on p. 233.

The volume will serve as an excellent introduction to industrial analysis, and will undoubtedly prove acceptable to all engaged in the teaching of analytical chemistry.

T. J. WARD.

INDUSTRIAL MICROBIOLOGY. THE UTILISATION OF BACTERIA, YEASTS AND MOLDS IN INDUSTRIAL PROCESSES. By HENRY FIELD SMYTH, M.D., Dr. P. H. and WALTER LORD OBOLD, M.S. London: Baillière, Tindall & Cox. 1930. Price 27s.

This book strikes rather a new note in bacteriological literature, for it is concerned entirely with the multitudinous fermentation reactions occurring in nature

and utilised in the arts and industries. It is in fact a compilation of almost every known fermentation process.

The industry of the authors in collecting references has been prodigious; there are no fewer than 675, many of them to patent specifications. Indeed, in this respect there is perhaps a weakness, for many of the patents described are of doubtful validity, and the processes covered of doubtful commercial utility. In some cases the authors themselves recognise this, as, for instance, in Chapter III, where, after describing a number of patents for the production of butyric acid, they use the very derisory phrase: "In brief, a compost pile of indiscriminate junk has been patented."

The book suffers a little at the outset by reason of a rather curious "General Introduction," which deals very briefly with some of the physical and chemical properties and requirements of micro-organisms. In this section such important matters as hydrogen-ion concentration and anaerobiosis are each dismissed in a few lines.

Each chapter is subdivided into a great number of short sections with cross headings, and this sometimes leads to curious results. Thus, on p. 30, the section headed Citric Acid begins: "In 1892 he described a particular group . . .," the pronoun referring to an author mentioned in the preceding Section.

The treatment of various subjects is rather unequal as to length and detail; a long chapter is devoted to butyl alcohol and acetone fermentation, but the production of casein is dealt with in ten lines. The statement that "whey . . . must be a by-product industry in cheese manufacturing to pay as a source of acid" is open to question, apart from its ungrammatical construction. It can hardly be said that lactic acid production from whey is as yet a profitable operation.

The authors feel that the policy of Prohibition in the United States makes a discussion of the preparation of fermented alcoholic beverages unnecessary, and they deliberately omit any reference to this important industry.

Chapter XII contains a valuable account of glycerin production; in Chapter XIII there is a very full description of the cultural characteristics of *Bacillus acetoethylicus*. Throughout the book the precise descriptions of organisms are an important feature.

There will be general agreement with the remark, referring to the use of Salmonella as destructors of rodents, that "the relation of epizootics in rodents to the public health of the community question (*sic*) the advisability of their general use."

Chapter XXVI, on Microbial Thermogenesis, is interesting. It is noticeable that little is really known about the spontaneous generation of heat in fermenting masses, *e.g.* in haystacks. It should be emphasised that very little reference is made to details of technique, but the value of the book lies in the precise descriptions of the various organisms best suited to specific fermentations, and, as a work

of reference in this particular field, it is undoubtedly of value to the technical bacteriologist. Anyone who wishes to know what products and by-products can be produced from any particular substance, and what organisms can be used to produce them, will find the information here.

The book concludes with a Chapter on Patent Law, contributed by J. Howard Flint, but this deals exclusively with United States Law, and is, therefore, of somewhat limited interest to British readers.

There is, naturally, a frequent use throughout the book of American turns of speech which sound strange to English ears, but, apart from the question of idiom, the English is by no means free from grammatical errors. Four misprints were noticed, one of which, "Poseudmonas" for "Pseudomonas," should not have escaped the proof reader.

R. F. HUNWICKE.

RECENT ADVANCES IN PHYSICAL AND INORGANIC CHEMISTRY. By ALFRED W. STEWART, D.Sc. Sixth edition. Pp. xi+388. London: Longmans, Green & Co. 1930. Price 18s. net.

The sixth edition of Prof. Stewart's well-known book is marked by the inclusion of five new chapters. A certain amount of the material which appeared in the earlier editions has been omitted, and the remainder of the text has been revised, wherever necessary, to bring it into line with modern developments.

The first thirteen chapters, occupying 205 pages, are devoted to "chemical physics," the subjects dealt with being line spectra and the Bohr theory, *X*-ray spectra, radioactivity, atomic numbers, isotopes and isobares, positive rays, the segregation of isotopes, the atomic nucleus and the electronic arrangement of the atoms. The section on line spectra and one dealing with cosmic rays are new to this edition. The second half of the book deals, in nine chapters, with topics more closely related to the ordinary work of the chemist, *i.e.* the recently discovered elements hafnium, illinium, rhenium and masurium, active hydrogen, hydrides, the Donnan equilibrium, flame reactions, emission band spectra, continuous emission spectra (the last four sections being new), and Tesla-luminescence spectra.

This book will appeal to the student who wishes to obtain information on these subjects. Prof. Stewart writes in a clear style which makes the reading of his book a pleasure as well as a source of profit; it would, for instance, be difficult to find a more lucid description of the elements of line spectra and the Bohr theory of the atom than that which appears in Chapter II. The book also supplies the demand of the student for information on subjects not usually found in the standard text-books, such as, for example, the preparation and properties of the hydrides.

In the reviewer's opinion, however, some of the material hardly conforms to the title "recent advances"; radioactivity, the radioactive emanations, Moseley's experiment and Aston's mass-spectrograph are subjects which have been assimilated into the body of chemical theory and find their place in the text-books.

On the other hand, the trend of modern quantum mechanics is of sufficient novelty and importance to deserve mention. The reviewer was disappointed to note the absence of any reference to recent developments in the theory of solutions; the name of Debye does not appear even once in the index. A chapter dealing with surface films and adsorption would also have been welcomed.

Apart from this, however, Prof. Stewart is to be congratulated on maintaining his high standard. The book is, with few exceptions, free from typographical errors, and the indexes are adequate.

R. A. ROBINSON.

NON-INTERPOLATING LOGARITHMS, COLOGARITHMS AND ANTILOGARITHMS. By FREDERICK W. JOHNSON, M.A. San Francisco: Simplified Series Publishing Co. 1930. Price $2.25.

The method of interpolation used in the usual form of logarithm table has been dispensed with in this book. The author has made use of the seven-place logarithms and antilogarithms of numbers, and by rounding them off has obtained tables accurate to the 5th and 4th figures, respectively. His method of arrangement permits one to read the figure required directly, no additions being necessary in order to arrive at the completed figure. The results obtained are for this reason more accurate than those given by the usual tables.

The marginal thumb index provided, together with the system of arrangement, ensure a saving of time that is further accentuated by the introduction of the table of cologarithms (logarithms of the reciprocals of numbers). The use of cologarithms makes it possible to solve fractions involving a number of factors in the numerator and denominator by the one operation of addition.

The average chemist, whose work rarely requires an accuracy involving more than 4 significant figures, will find the usual 4-figure mathematical tables more convenient for his purpose. The physicist, statistician and others using 5-figure logarithms with sufficient frequency, will find this work of value.

A table of International Atomic Weights is given, with the logarithm and cologarithm of each. Tables of certain of the more frequently used chemical, physical and mathematical constants are provided, together with a useful table of metric conversion factors; in all cases the logarithm and cologarithm are given.

In addition, use is made of the functions, S, T, etc., which represent

$$\log \frac{\sin x}{x \text{ (in seconds of arc)}}, \quad \log \frac{\tan x}{x},$$

etc., respectively, for accuracy in the determination of the trigonometrical functions of small angles and angles approaching 90°.

There is a well-written, lucid introduction explaining the use of the various tables.

M. BOGOD.