THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 5.30 p.m., on Wednesday, May 1st, 1940, at the Chemical Society's Rooms, Burlington House, the President, Dr. E. B. Hughes, in the chair.

Sir William Willcox, K.C.I.E., C.B., C.M.G., F.I.C., was elected an Honorary Member of the Society, and J. A. Freeman, B.Pharm., B.Sc., F.I.C., Ph.D., M.P.S., and J. T. Stock, B.Sc., were elected Ordinary Members.

Applications for membership were read in favour of J. N. Davidson, B.Sc., M.D., J. R. Fraser, B.Sc., F.I.C., G. N. Gee, D. E. Jones, M.Sc., F.I.C., F. L. Kinsella, R. K. Matthews, F.I.C., W. J. Puregger, R. E. Stuckey, B.Sc., A.I.C., Ph.C., and J. A. C. Watson, A.I.C.

The following papers were presented and discussed:—"The Spectrophotometric Assay of Vitamin A, with special reference to Margarine," by J. R. Edisbury, B.Sc., Ph.D.; "The Estimation of Vitamin D in Margarine," by N. T. Gridgeman, B.Sc., H. Lees, B.Sc., and H. Wilkinson, B.Sc., Ph.D.; "The Composition of some Jam Fruits and the Determination of the Fruit Content of Jams," by T. Macara, F.I.C., and C. L. Hinton, F.I.C.

NORTH OF ENGLAND SECTION

A MEETING of the Section was held in Manchester on April 13th, 1940. The Chairman (Mr. J. R. Stubbs) presided over an attendance of thirty, which included the President (Dr. E. B. Hughes).

The following papers were read and discussed:—"The Iodimetric Determination of Alkali—An Investigation of Smith's Proposed Method," by J. Haslam, M.Sc., F.I.C., and R. F. Roberts; "Note on an Unusual Case of Cattle Poisoning," by J. G. Sherratt, B.Sc., F.I.C.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held in the North British Station Hotel Edinburgh, on Friday, April 19th, at 6.30 p.m.

The following papers were read and discussed:—"Notes on the use of Selective Oxidation in the Analysis of Fats," by W. A. Alexander, B.Sc., A.I.C.; "The Determination of Ketone Bodies in Body Fluids." "The Determination of Magnesium in Body Fluids," by H. Dryerre, Ph.D., M.R.C.S., L.R.C.P.; "The Pollution of Water Supplies by Trout and Gulls," by A. Dargie, B.Sc., A.I.C.

Obituary

SIR GILBERT THOMAS MORGAN, F.R.S.

THE death of Sir Gilbert Morgan on February 1st has removed one of the most distinguished of British chemists, and our Society deplores the loss of one of its Honorary Members. Many of us deeply regret the passing of an old and valued friend of many years standing, a man to whom one could go for sound advice and with whom one could talk freely on chemical and more mundane matters.

Born in 1870, Morgan was educated at the Central Foundation School and then at the Finsbury Technical College, where so many of our chemical friends received their scientific training. At that time the head of the Chemical Department was Professor Meldola, and the routine laboratory work was in the charge of J. Castell Evans and F. W. Streatfeild. Meldola had left the dyestuff industry for an academic career, and the research work at Finsbury was confined almost entirely to aromatic compounds containing nitrogen. Morgan assisted Meldola and Streatfeild in their work on the structure of the diazoamino-compounds, and after leaving College spent some time in the works of Read, Holliday & Co., at Huddersfield.

Returning to London, he was a demonstrator and then Assistant Professor at the Royal College of Science, where, at that time, Tilden was Professor of Chemistry. Always a great worker, Morgan found time to act as an abstractor, and afterwards edited the *Transactions of the Chemical Society* from 1903 to 1906.

Research work occupied all his spare time, and with the Finsbury tradition and the subsequent works experience it is no wonder that his earlier independent work was chiefly confined to aromatic compounds containing nitrogen. In conjunction with several co-workers, especially Miss F. M. G. Micklethwait, he published a series of papers, several of which dealt with diazoamino compounds and non-aromatic diazonium salts; the latter continued to interest him for some time after he left South Kensington. Morgan's interest was also aroused by the "residual affinity" of coumarin in forming salts with inorganic acids, and further by organic compounds in which arsenic and antimony were directly linked with carbon; both of these interests led to a large output of research in later years.

From 1912 to 1915 Morgan was Professor at the Royal College of Science, Dublin, and after a short period at British Dyestuffs, Ltd., succeeded Meldola as Professor of Chemistry at Finsbury Technical College, where he remained from 1916 to 1919. Publication was necessarily restricted during the war years, as he was engaged in matters of immediate importance for the country. His advice, meanwhile, was at the disposal of others, and the present writer had particular reason for gratitude in the matter of an autoclave of laboratory dimensions which was safe to a reasonably high pressure. Such articles were not often found in scientific laboratories in 1914!

In 1919 he became Mason Professor of Chemistry at Birmingham University, and a time of great activity followed. A crowd of enthusiasts was collected and worked from morning to night; even lunch was taken in the laboratory, and Morgan and his co-workers ate, drank coffee and "talked shop." The present writer has a happy memory of a casual visit to Birmingham and a lunch with these energetic chemists.

Arsenic and antimony linked to carbon had given another direction to Morgan's work and, not unnaturally, the corresponding sixth-group elements, selenium and tellurium, were soon under examination. Moreover, the "residual affinity" question had interested him in co-ordination, with the result that the different lines of research became merged and a large amount of work on metallic derivatives of various organic compounds was the result. The writer believes he is not mistaken in attributing the term "chelation" to Morgan.

In 1925 Morgan became Director of Chemical Research (D.S.I.R.), and took charge of the Chemical Research Laboratory at Teddington. His manifold chemical interests and energy suited him admirably for this post; the variety of work was great, so was the output of research. Low-temperature tars, reactions under high pressure, chemotherapy, synthetic resins are only some of the subjects which were dealt with in the laboratory. In one of his presidential addresses to the Chemical Society, he gave an account of some of the chief lines of research in which the laboratory had been engaged, and, incidentally, his hearers learnt many facts of chemical value. This presidential address was of outstanding interest.

Besides work of obviously practical application, research on the purely scientific side of the many problems involved was continued, and a glance through the indexes of *British Chemical Abstracts* gives some idea of Morgan's many-sided activities. He also found time to contribute articles to *Thorpe's Dictionary of Applied Chemistry*, and he edited the chemistry section of the *Encyclopaedia Britannica* (1929), besides publishing books on *Organic Compounds of Arsenic and Antimony* (1918), and *Inorganic Chemistry* (with Burstall, 1936).

On retiring from Teddington, where he is succeeded by a former co-worker, Dr. G. S. Whitby, he became Research Director of the Institute of Brewing; this post he held until his death.

From 1910 to 1912 Morgan was Hon. Secretary of the Chemical Society, and President from 1931 to 1932. He was also President of the Society of Chemical Industry in 1931–1932, and received the Society's medal in 1939; for some years he was Chairman of the Bureau of Chemical Abstracts, and was so at his death.

He graduated as D.Sc. (Lond.) and held various honorary degrees; Sc.D. (Dubl.), LL.D. (Birm., St. And.), M.Sc. (Birm.), and he became F.R.S. in 1915. In addition to his Honorary Membership of our Society, he was a Corresponding Member of the Royal Dublin Society, Honorary Associate of the Manchester College of Technology, F.I.C. and F.C.G.I. In recognition of his war work he was made O.B.E. in 1920, and this was followed by a knighthood in 1936.

Morgan will be sadly missed by his many friends, who remember his kindly acts and recall a humorous and lively companion. Those who did not know him so well personally need only talk to his former students and co-workers (many of whom are already well known in the chemical world) to realise the affection and esteem in which he was held.

J. T. HEWITT

JOHN WHITE, F.I.C.

THE death of John White, on March 30th, in his seventy-seventh year, makes another gap in the now very thin rank of those whose membership of the Society dates back to last century.

White was born at West Bromwich and, after studying chemistry for three years at the Birmingham and Midland Institute, he became a pupil and, later, chief assistant in the laboratory of the late Dr. Bostock Hill. During this time he was appointed Public Analyst for his native town of West Bromwich, carrying on his work in Hill's laboratory until, in 1894, he was appointed "whole time" Public Analyst for the County of Derby, and carried on the duties of this appointment until his retirement in 1933. He was also Public Analyst for the County Borough of Derby, and the Borough of Glossop, Official Agricultural Analyst and Water Examiner for the County and Borough of Derby, Consulting Chemist to the Glossop Sewage Works and to the Derbyshire Agricultural Society, and Gas Examiner for most of the Boroughs and Urban and Rural District Councils within the county.

He was elected a member of this Society in 1893, served as ordinary member of the Council during various periods ranging from 1898 to 1926, and also acted as

Vice-President in 1907–8, and again in 1927–28. He became an Associate of the Institute of Chemistry in 1888 and a Fellow in 1891, and served on its Council in 1910–13. He was for some time Chairman of the Nottingham Section of the Society of Chemical Industry.

White, to those who, like the writer of these notes, knew him intimately, was a man of endearing personality. Under a modest and retiring exterior, he had a wide knowledge of general science, with a supreme sense of public duty and a corresponding zeal for carrying it out with the wise caution of a well-balanced mind. During his earlier days he rarely missed a meeting of our Society, and was always helpful in discussion, although he did not contribute many original papers to our proceedings. Among the few was an interesting paper on "The Occurrence of Barium Compounds in Artesian Well Waters" (ANALYST, 1899, **24**, 67); others were on the "Use of Maize as an Adulterant of Oatmeal" (ANALYST, 1895, **20**, 30), on "Caper Tea" (ANALYST, 1899, **24**, 117), and on "Spurious Cream of Tartar" (ANALYST, 1902, **27**, 118).

The writer has pleasant recollections of a Summer Meeting of the Society at Derby during his own Presidency in 1897, arranged by White, aided by our late President, Leonard Archbutt (Chemist to the Midland Railway), on which occasion we were shown over the laboratory and locomotive works of the Railway, the printing and lithographic works of Messrs. Bemrose & Sons, and the works of the Crown Derby Porcelain Manufactory. Among the few survivors of the happy party assembled on that occasion is our old member A. H. M. Muter, still happily active, whose membership dates from the same year (1893) as that of White.

The hobby in which White found change and relaxation from his strenuous work was golf, in which sport he was a creditable performer. For many years he and Archbutt played regularly together on their home links, and the writer well remembers occasional days on links nearer to London, when White joined in "foursomes" with him and Bevan and Clowes or Oscar Guttmann—days stolen by White on the morrow of Society meetings.

On his retirement from office White went to live at Weston-super-Mare, but moved later to Sutton Coldfield, where he died. He was buried at Derby, his funeral service being conducted by the Ven. Dr. Noakes, Archdeacon of Derby, to whom at one time he had been churchwarden at St. John's Church, Derby.

He leaves a widow, a son and two daughters, one of whom is the wife of our member Mr. Stanley Dixon, Public Analyst for Cardiff. BERNARD DYER

Biological Assay of Vitamin D_3

I. Assay Methods at Present in Use, with Particular Reference to Olsson's Radiographic Technique

BY A. Z. BAKER, B.Sc., M.R.C.S., L.R.C.P., AND M. D. WRIGHT, B.A., M.B., B.S.

EVER since the discovery[1,2] of the nutritional importance of animal fats containing fat-soluble vitamin D for the normal calcification of bone, methods of measuring the extent of calcification have been made the basis for estimating the amount of the antirachitic vitamin present in cod-liver oils, etc.

Both the rat and the chick have been extensively used as the test animal for this purpose. Work on chicks was at first hindered by the practical difficulties associated with rearing day-old birds under laboratory conditions. Most of the chick studies are by American workers, a large number of whom have approached

the question of calcification in the bird chiefly from the standpoint of sound practice in poultry rearing. So far, the best-developed method has been the use of the percentage ash-content of the tibia determined under standard conditions.[3] It is well recognised that experimental rickets in the bird, unlike its analogue in rats, can be produced without gross disturbance of the mineral balance of the ration, although favourable Ca:P ratios in the diet exert a sparing effect on the vitamin D needed for calcification to proceed normally. A considerable body of information has been accumulated about the influence of other dietary constituents upon the chick's requirement of the calcifying vitamin, the cereal,[4] and especially the mineral composition of the ration, having been subjected to much study.

In this country the rat has been the experimental subject for vitamin D assay almost to the exclusion of the chick. All the methods in use, however, measure the extent of bone development, with the proviso that experimental rickets in rats demands for its appearance a highly abnormal mineral balance in the diet. The three methods used hitherto for the study of bone structure are (*a*) McCollum's line test[5] based on the cure of rickets; (*b*) estimation of the ash-content of the bones[6] of the hind limbs of rats maintained on prophylactic levels of vitamin D; (*c*) radiographic examination of rats undergoing either curative or prophylactic tests.[7] In addition, a method has been described based upon the increase of weight observed in rats on a supplement of irradiated ergosterol ;[8] but the method, though an actual measure of the vitamin activity, did not show sufficient accuracy to justify its further use.

Much of the earlier work on the chick was concerned with finding the percentage of cod-liver oil which would afford protection from rickets under any given conditions of diet, but the discovery in 1926[9,10] that vitamin D can be produced by irradiation of ergosterol led to the establishment of irradiated ergosterol as the standard substance for vitamin D assays. The unit of activity was taken as 1 mg. of a standard solution of the material in olive oil, and this unit was adopted by the Pharmaceutical Society in 1927, the Medical Research Council in 1930, and the International Vitamin Conferences of 1931 and 1934.

With the adoption of a vitamin D standard, progress increased. Many of the difficulties inherent in the interpretation of results from animal observations made without reference to a standard substance were controlled and technique was improved. Routine assays of cod-liver oil quickly came to be regarded as essential for examining oils bought and sold with a vitamin D guarantee. Rat methods remained in almost exclusive use in this country, but most American stations used the A.O.A.C. method of comparing oils for agricultural feeding with a standard cod-liver oil.

Anomalous differences in the behaviour of rats and chicks soon became apparent. Rickets appeared when trials were made to protect chicks by feeding to them an amount of irradiated ergosterol equivalent in "rat units" to an amount of cod-liver oil that would have afforded ample protection. In 1928,[11] it was stated that 0·0001 mg. of irradiated ergosterol daily would cure rickets in the rat, but that the chick needed as much as 0·01 mg., and that its immunity from overdose of ergosterol was exceedingly high. In 1930, Massengale and Nussmeier[12] studied

the chick requirement of irradiated ergosterol by two methods:—determining the calcium and phosphorus in the serum and the bone ash in the femur. They found that approximately 100 times the number of rat units must be fed as the irradiated product to give a mean value for the percentage of ash in groups of 10 birds as high as was given by 2 per cent. of cod-liver oil in the basal ration. This high requirement of the chick for irradiated ergosterol was widely confirmed in the next few years. Steenbock and his co-workers in 1932[13] quoted it as between 40 and 120 times the number of rat units that would be sufficient as cod-liver oil, and came to the conclusion that the vitamin D produced by ordinary irradiation of ergosterol is a different substance from the antirachitic factor in cod-liver oil.

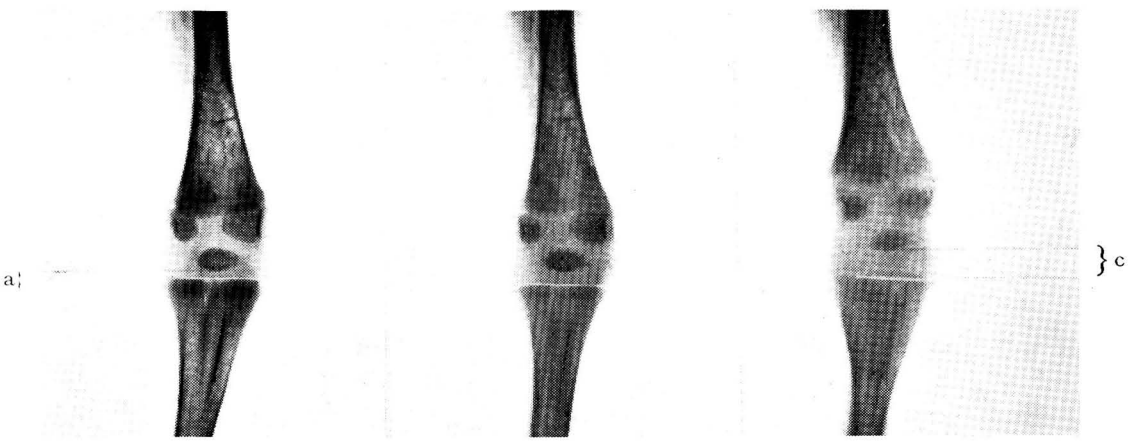
The ultimate discovery of vitamin D_3 was approached by deduction from the observation made by several investigators that, although irradiated ergosterol was so inactive in the chick, exposure of the bird to very short periods of irradiation was highly protective.[14,15,16] Waddell (1934)[17] therefore irradiated an animal extract composed of crude cholesterol, lecithin and fat and found that it was quite as effective for chicks as cod-liver oil, fed in equivalent "rat units." Since then, vitamin D_3 obtained by irradiating 7-dehydrocholesterol has been found to give the same result; it has been isolated, and its constitution has been established.

The calcifying vitamin effective for birds is thus available in pure form as a standard against which to assay oils for poultry feeding. No such standard has yet come into use, and the custom of expressing the vitamin D potency of oils for poultry feeding in International Units as a result of rat assay, using as the standard the official solution of irradiated ergosterol, is still widespread. It should be understood that this test measures the calciferol or vitamin D_2 activity and does not necessarily indicate an equal potency of vitamin D_3 in an oil. The vitamin D_2-like activity of substances is not a reliable indication of their vitamin D_3-like activity, that is, of their efficiency for the chick.

Olsson, in 1936,[18] reported that the amount of vitamin D_3 in chick rations could be related, within certain limits, to the width of the tarso-metatarsal (TMT) distance measured in radiographs of the growing bones (Fig. 1). He considered this a sensitive and satisfactory assay method (Fig. 2, p. 329). Work to confirm and expand his findings has been in progress for the last 18 months in these laboratories; in the earlier tests a provisional standard reference oil, BZ1, which had been used in an extensive series of tests on rats, and was kindly provided by British Colloids, Ltd., was employed. This oil was suitably stored and fed in graded amounts in a series of chick tests, with reasonably uniform results. More recently it has been possible to obtain, through the British Standards Institution (Vitamin D Panel of Sub-committee C 11/2: Chick Test for Veterinary Cod-liver Oil) supplies of pure vitamin D_3 in solution for use as a standard for direct comparison.

Olsson's finding, that the width of the TMT distance can be used as a basis of vitamin D_3 assay, has been confirmed but, following a suggestion based on statistical analyses by Mr. E. C. Fieller, it has been found that the relation is more accurately expressed by comparing the logarithm of the TMT distance,

Fig. 1



(a) (b) (c)

Tarso-metatarsal distance

(a) Normal (b) Slight degree of rickets (c) Severe degree of rickets

rather than the distance itself, with the logarithm of the dose. The first table below, however, is based on Olsson's method of expression.

The principles of Olsson's radiographic method have been used in our tests, but with slight differences in some particulars, which will be described. Olsson at first recommended 6 weeks as the optimum age for radiographic examination, but in a later paper[19] he suggests 4 to 6 weeks. We have taken X-ray photographs at 4, 5 and 6 weeks and have recorded most of the results at 5 weeks. This period

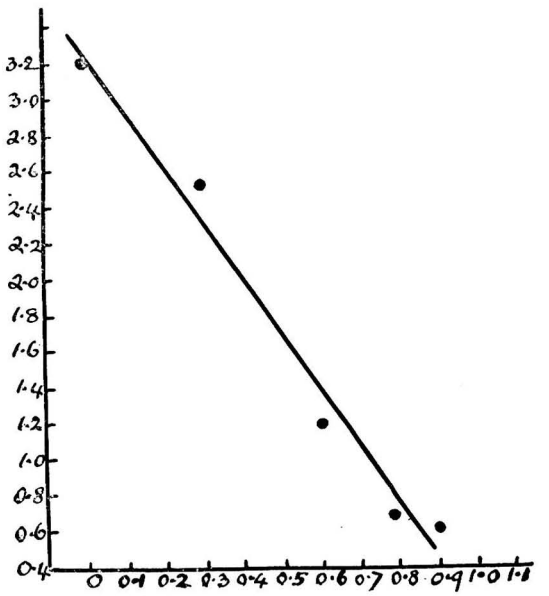


Fig. 2

was chosen in preference to 4 weeks, as the slightly heavier bones yielded radiographs rather more easily measured than those obtained at 4 weeks of age. The possible increase in accuracy by using data obtained after 5 weeks' feeding, following a one-week depletion period, is under investigation.

TABLE I

Level of oil fed	Mean TMT		Standard deviation	
	4 weeks	6 weeks	4 weeks	6 weeks
Per Cent.	mm.	mm.		
0·50	1·35	1·27	0·164	0·159
0·25	1·37	1·31	0·131	0·181
0·125	1·50	1·43	0·111	0·185
0·0625	1·88	1·74	0·596	0·485

TECHNIQUE.—*Chicks Used.*—These are sex-linked cockerels (Light Sussex and Rhode Island Red), obtained as day-old chicks from a known reliable source.

Method of Housing.—The chicks are reared, 20 in a compartment (dimensions 10″ × 11″ × 66″) in Summit Morcote all-metal brooders specially modified by Messrs. Cope & Cope, Reading, for our purpose. Each brooder contains 6 units in

three rows of two and each unit contains a warm chamber and an unheated run. The floors are of wire mesh over metal trays. Heating is by electricity, the element being placed in the roof of the closed portion and the temperature thermostatically controlled. The battery of brooders is housed in a brick building, from which daylight is entirely excluded by insulated shutters, light being supplied during 12 hours a day by electric lights so placed as to give even illumination to all the compartments. Each unit is provided with a trough containing water kept at a constant level; this prevents crowding.

Ventilation is effected by baffled inlets near floor level, the outlets being along a central ceiling shaft communicating with an exhaust fan of controlled speed.

Food.—The chicks are all given a basal ration to which are added different quantities of the test oil in olive oil, to make a total addition of 1 per cent. of oil. The *basal* ration is as follows:—maize meal, 35; middlings, 24; bran, 16; dried skimmed milk, 8; meat meal (albumenoids 50 per cent.), 10; dried grass, 5; salt, 0·5; ground limestone, 1·5; olive oil, 1·0 per cent. The grass meal was included for its high carotene content; its use made it unnecessary otherwise to modify the vitamin A contents of the different diets. *Analysis.*—protein, 18·19; fat, 5·27; ash, 6·85; ash, calcium, 1·82; phosphorus, 0·98; fibre, 4·7 per cent. Ca/P = 1·85; vitamin A and carotene, 210 I.U. per 100 g.

The ingredients are weighed on a balance recording down to 0·25 oz. and mixed in an electric mixer for 20 minutes. A portion is then removed, the oil is mixed with it by hand and the food is sieved, after which it is returned to the bulk and mixed for a further 20 minutes. Determinations of oil in random samples of mashes prepared in this way have shown that such mixtures are uniform.

Depletion Period.—In recent tests the birds have been reared for the first week on the basal ration without vitamin D supplement in order to exhaust their yolk sac store and give the greatest possible uniformity among those selected for the test.

Treatment of the Test Oils.—The uniform admixture of the test oils and olive oil is also effected by electric stirring in a Hobart machine. The basal diet used in conjunction with an adequate supply of cod-liver oil produces sturdy well-feathered chicks, weighing approximately 10 oz. at 4 weeks and 17 to 20 oz. at 6 weeks.

Procedure.—At first 40 chicks were fed at each level of any test oil, usually in 2 units of 20 each. A number of oils are fed simultaneously, and the groups are so arranged as to reduce as far as possible differences which might arise from different positions in the house. More recently the allocation of groups of 20 birds at four or more levels of the standard and of the test oil respectively has made it possible to assay with considerable accuracy, and with economy of time, samples of widely differing vitamin activity. The value of using several levels under these conditions is stressed, for this provides a good "spread" from which to calculate the slope of the curve of response.

X-ray Technique and Records.—The tarso-metatarsal joints are photographed in an anteroposterior position, an exposure of 0·25 second at 12 inches, 12 ma. Position 2 Kv (Victor X-ray apparatus) being given. The tarso-metatarsal distance is measured on the film (supported on a viewing screen) by a vernier microscope magnifying 5 diameters (Baker) and reading to 0·02 mm. Olsson's

method of magnifying 8 to 12 diameters has not been found possible on the films available in this country, owing to the size of grain.

Accuracy has been increased by the following slight modification of Olsson's technique, suggested by Mr. E. T. Halnan of the School of Agriculture, Cambridge. A fine line is drawn with a razor blade tangential to the proximal end of the fused metatarsus, and the distance is taken between this line and the nearest point of the central tarsal bone. By this modification the range of one group of figures was reduced from 0·62–1·56 to 0·78–1·02 mm., and that of another from 1·34–4·88 to 1·52–4·52 mm.

Results.—The method has been used with several different samples of cod-liver oil, with various fish oils of high potency and with cod-liver oil concentrates, and we are able to confirm Olsson's finding that it is accurate and economical. The modification of using log TMT, rather than the distance itself, was introduced to equalise the variability of responses to different doses.

TABLE II

	Test dose per 30 g. of mash cg.	No. of chicks	Log of mean response, mm. × 10	σ	Coefficient of variation
I	4	17	0·938	0·120	12·8
II	3	18	1·132	0·163	14·4
III	2	16	1·252	0·177	14·1
IV	1	16	1·396	0·163	17·7

On the scale on which the work is done in these laboratories it has been found possible, after practice, to evaluate the results graphically to a figure within 5 to 8 per cent. of that obtained by full statistical analysis; however, this has been carried out in every test to obtain the error of each assay and to detect and assess variations from linearity.

Statistical analysis is of rather greater importance when the scale of the work is more limited than is described in this paper, and the additional information thereby obtained will generally amply repay the labour involved. The method used for analysis is set out in the appendix.

Many oils have been examined in these laboratories by the radiographic method, and the following are typical of the results obtained:

TABLE III

Cod-liver oil	Value u.p.g.	No. of chicks	Percentage limits of error P = 0·95	P = 0·99	
Sample A	65	81	80–126	74–135	Standard reference oil, C.L.O., taken
B	82	60	74–135	68–148	as 200 units per g. (assayed on rats)
C	131	63	84–119	80–126	,, ,, ,,
D	100	80	84–119	79–126	,, ,, ,,
E	70	70	84–119	79–126	,, ,, ,,
F	89	84	83–121	78–129	,, ,, ,,
G	111	76	86–117	81–123	,, ,, ,,
H	97	76	85–118	81–124	,, ,, ,,
I	46	67	80·6–124	75·5–132·5	Pure vitamin D_3

Samples of other liver oils at suitable dilutions have also been examined with comparable results.

TABLE IV

High potency oils	Value u.p.g.	No. of chicks	Percentage limits of error P = 0·95	P = 0·99	
Sample A	35,000	114	85–117	81–123	Standard reference oil, C.L.O.
B	3,400	65	82–122	77–130	,, ,, ,,
C	5,500	76	74–135	68–148	,, ,, ,,
D	14,300	54	90–111	87–115	,, ,, ,,
E	11,080	49	80·6–124	75·5–132	Pure vitamin D_3

TABLE V

Method	Standard deviation	Slope	No. of chicks receiving any one oil	Percentage limits of error P = 0·95	P = 0·99
Radiographic ..	0·293	−1·29	200	93–107	91–110
			100	90–111	88–114
			50	86–116	83–121
			20	79–126	74–135
			10	72–138	65–153
Bone ash, per cent. ..	2·364	8·672	200	92–109	89–112
			100	88–113	85–118
			50	84–119	79–126
			20	76–132	69–144
			10	67–148	60–167

Some comparisons have been made between the error given by the radiographic chick method and that obtained with birds on the same doses of the oils under examination, tested by Dr. Magnus Pyke of these laboratories, under the standard conditions laid down by the Association of Official Agricultural Chemists (U.S.A.), but with a separate determination of the ash percentage of each bone. Figures from such a test are given in Table V, from which it is clear that experimental error is of the same order with Olsson's technique as in the A.O.A.C. method. The radiographic method avoids certain disadvantages inseparable from the A.O.A.C. technique, *viz.*: (1) The necessity for killing the birds in order to collect the experimental data. (2) The long time consumed in dissecting the bones and the difficulty of reducing this beyond certain limits, for the accuracy of the results depends to a considerable extent upon skilful and uniform dissection of all the samples. Unless individual bones are separately ashed, the error of any experiment cannot be stated. Group ashing has been widely practised in assays by the A.O.A.C. technique.

The main practical advantages of the radiographic technique are: (1) That a permanent record of assays can be kept for reference at any future date. The X-ray photographs can be measured by different workers, if desired, and allowance made for any individual differences. (2) That the chicks are rearable after the experiment, so that larger numbers can be used without a corresponding increase in expense. Greater accuracy is thus obtained at lower cost than in the A.O.A.C. method. In this laboratory 840 chicks are used at one time for a series of tests and are later reared under farm conditions for the table.

SUMMARY.—1. Since normal osteogenesis in birds is promoted far more economically by vitamin D_3 than by calciferol, stress is laid on the practical importance of basing assays of the antirachitic value of liver oils for poultry feeding upon bird tests, using pure vitamin D_3 as the standard of reference.

2. Olsson's technique has been investigated and modified. Confirmation has been obtained of his claim that the TMT distance in chicks is a function of the logarithm of the amount of vitamin D_3 supplied in the diet, but it is found that using the logarithm of the TMT gives greater accuracy than using the actual distance.

3. The error of the Olsson method has been estimated in a number of assays and found to be approximately the same as that of the A.O.A.C. method conducted in the same laboratory.

4. The Olsson method has been found to be of relatively low cost in laboratories where a suitable X-ray plant is available.

Our thanks are due to Mr. E. C. Fieller, for the statistical treatment of assays and for his helpful criticism and advice; also to Mr. L. Jones, of British Colloids, Ltd., for duplicating the ash determinations.

APPENDIX.—This appendix illustrates the method of statistical analysis used in the vitamin D assays of oils. In a typical assay readings were obtained from groups of 17, 18, 16, 19 and 18 chicks, which had received supplements of 5, 10, 15, 20 and 30 units of standard oil per 100 g. of food respectively. Table A, column (2) gives the logarithm of the dose in units/20 g. of food, and column (3) the number of readings per group. Column (5) gives the sum of the responses, which are recorded as [1 + $\log_{10}$ (TMT distance in mm.)], in the successive groups, and column (10) the sum of their squares.

TABLE A

Dose (1) u/100 g.	(2) x_1	(3) n_1	(4) n_1x_1	(5) n_1y_1	(6) y_1	(7) $n_1x_1y_1$	(8) $n_1x_1^2$	(9) $n_1y_1^2$	(10) Σy_1^2	(11) $f_1s_1^2$	(12) f_1	(13) s_1^2
5	0·0000	17	0·0000	24·33	1·431	0·000000	0·000000	34·8205	35·2185	0·3980	16	0·02487
10	0·3010	18	5·4180	21·76	1·209	6·549760	1·630818	26·3054	25·6660	0·3606	17	0·02121
15	0·4771	16	7·6336	16·84	1·052	8·034364	3·641991	17·7241	18·3076	0·5835	15	0·03890
20	0·6021	19	11·4399	19·19	1·010	11·554399	6·887964	19·3819	19·9687	0·5868	18	0·03260
30	0·7782	18	14·0076	16·94	0·941	13·182708	10·900714	15·9424	16·3296	0·3872	17	0·02278
Sums (Σ)		88	38·4991	99·06		39·321131	23·061487	114·1743	116·4904	2·3161	83	
			Correction terms	..	..	43·337737	16·842963	111·5100				
			Differences	..	..	−4·016606	6·218524	2·6643				

Column (6) contains the mean responses, and column (9) their products by the group sum. By subtracting this from the entry in column (10), we obtain the sum of the squares of the deviations of the individual responses in each dosage group from their mean (column 11). Column (12) gives the corresponding degrees of freedom (group frequency less one); dividing this into the entry in column (11) we obtain the estimated variance of individual responses to the successive doses (13). These variances, and those for the dosage groups on the test oil, prove to be in good agreement with each other, and we are therefore justified in pooling them and in evaluating the assay by the method described by Irwin.[20]

The calculations for the slope of the log (dose)-response line are contained in columns (7) and (8), which are explained by their headings; the correction terms subtracted from their totals are obtained from the totals of columns (3), (4) and (5) as $(\Sigma nx)\ (\Sigma ny)/(\Sigma n)$ and $(\Sigma nx)^2/(\Sigma n)$. For later use we also subtract a term $(\Sigma ny)^2/(\Sigma n)$ from the total of column (9).

The test oil was fed at levels of 1, 2, 3 and 4 cg./32 g. of food, and similar calculations to those in Table A lead to the totals given in Table B (x_2 = log. of dose in cg./32 g. of food).

TABLE B

	n_2	n_2x_2	n_2y_2	$n_2x_2y_2$	$n_2x_2^2$	$n_2y_2^2$	Σy_2^2	$f_2s_2^2$	f_2
Sums (Σ)	67	23·6395	79·68	25·345031	11·709770	94·2655	95·8172	1·5517	63
Correction terms		..	..	27·760534	8·340686	92·3962			
Differences	..	..	..	−2·415503	3·369084	1·8693			

We now calculate the means

$$\bar{x}_1 = 38{\cdot}4991/88 = 0{\cdot}4375 \qquad \bar{y}_1 = 99{\cdot}06/88 = 1{\cdot}1257$$

$$\bar{x}_2 = 23{\cdot}6395/67 = 0{\cdot}3528 \qquad \bar{y}_2 = 79{\cdot}68/67 = 1{\cdot}1743$$

and the slope

$$b = -\frac{4{\cdot}0166 + 2{\cdot}4155}{6{\cdot}2185 + 3{\cdot}3691} = -\frac{6{\cdot}4321}{9{\cdot}5876} = -0{\cdot}6709$$

The log. (potency ratio) is estimated to be

$$M = (\bar{x}_1 - \bar{x}_2) - (\bar{y}_1 - \bar{y}_2)/b = 0{\cdot}012151 = \log.\ (1{\cdot}0285).$$

We therefore estimate that 31·25 mg. of the test oil contain $5 \times 1{\cdot}0285 = 5{\cdot}143$ units of D_3, *i.e.* that its potency is 164·6 u/g. To verify that the series of points (x, y) do not deviate significantly from straight lines, we calculate the sum of squares (for 6 degrees of freedom)

$$2{\cdot}6643 + 1{\cdot}8693 - \frac{(6{\cdot}4321)^2}{9{\cdot}5876} = 0{\cdot}2184$$

and compare the corresponding mean square, 0·0364, with the pooled group variance (with 146 degrees of freedom)

$$s^2 = (2{\cdot}3161 + 1{\cdot}5517)/(83 + 63) = 0{\cdot}02649.$$

Since s^2 is not significantly smaller than the mean square 0·0364, the linearity of the log (dose) response relation is verified, and we can calculate the approximate error of M from Irwin's formula:

$$\sigma_M^2 = \frac{s^2}{b^2}\left\{\frac{1}{\Sigma n_1} + \frac{1}{\Sigma n_2} + \frac{(\bar{y}_1 - \bar{y}_2)^2}{b^2\{\Sigma n_1(x_1 - \bar{x}_1)^2 + \Sigma n_2(x_2 - \bar{x}_2)^2\}}\right\}$$

$$= \frac{0{\cdot}026492}{0{\cdot}450076}\ (0{\cdot}011364 + 0{\cdot}014925 + 0{\cdot}000540) = 0{\cdot}0015797$$

Whence $\sigma_M = 0{\cdot}03975$.

For 146 degrees of freedom, the 5 per cent. and 1 per cent. levels of Student's t are 1·977 and 2·610. Since $1{\cdot}977\sigma_M = 0{\cdot}0786$, $2{\cdot}610\sigma_M = 0{\cdot}1037$, the limits of M are

for P = 0·95 : 0·0122 ± 0·0786, *i.e.* $\bar{1}{\cdot}9336$ and 0·0908,

for P = 0·99 : 0·0122 ± 0·1037, *i.e.* $\bar{1}{\cdot}9085$ and 0·1159.

The limits for the D_3 content of 31·25 mg. of test oil are therefore

for P = 0·95 : 5(0·858) units to 5(1·233) units,

for P = 0·99 : 5(0·810) units to 5(1·306) units.

and those for the content of 1 g. of test oil

for P = 0·95 : 137 units to 197 units,

for P = 0·99 : 130 units to 209 units.

REFERENCES

1. E. Mellanby, *J. Physiol.*, 1918, **52**, XL.
2. V. Korenchevsky, 1922, *Special Report Series*, Medical Research Council, London, No. 71.
3. *A.O.A.C. Methods*, 1937, **20**, I, 72.
4. H. D. Branion, *Poultry Science*, 1932, **12**, 335.
5. E. V. McCollum, N. Simmonds, P. G. Shipley and E. A. Parks, *J. Biol. Chem.*, 1922, **51**, 41.
6. H. Steenbock and A. Black, *J. Biol. Chem.*, 1924, **61**, 405.
7. R. B. Bourdillon, H. M. Bruce, C. Fischmann and T. A. Webster, 1931, *Special Report Series*, Medical Research Council, London, No. 158.
8. K. H. Coward, K. M. Key and G. E. Morgan, *Biochem. J.*, 1932, **26**, 1585.
9. O. Rosenheim and T. A. Webster, *id.*, 1927, **21**, 389.
10. A. Windaus and A. F. Hess, *Nach. Ges. Wiss.*, Göttingen, 1926, **2**, 175.
11. H. Kreitmair and T. Moll, *Munch. Med. Woch.*, 1928, **75**, 637.

12. O. N. Massengale and M. Nussmeier, *J. Biol. Chem.*, 1930, **87**, 423.
13. H. Steenbock, S. W. F. Kletzien and J. G. Halpin, *id.*, 1932, **97**, 249.
14. H. T. Scott, E. B. Hart and J. G. Halpin, *Poultry Science*, 1929, **9**, 65.
15. W. C. Russell, O. N. Massengale and C. H. Howard, *J. Biol. Chem.*, 1928, **80**, 155.
16. W. C. Russell and C. H. Howard, *id.*, 1931, **91**, 493.
17. J. Waddell, *id.*, 1934, **105**, 711.
18. N. Olsson, *Arch. fur. Geflügelkinde*, 1936, **10**, 11–12.
19. —— *Kungl. Fyriografiska Sällskapets i Lund Forhandlingar*, 1936, **9**, 1.
20. J. C. Irwin, Statistical Method Applied to Biological Assays, Supp. to *Trans. R.S.S.*, 1937, IV, 1–48.

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A New Group Separation for the Quantitative and Qualitative Analysis of Phosphates

BY G. J. AUSTIN

HITHERTO no method of analysis suitable for the quantitative separation of the common metals has been applicable in presence of more phosphate than is equivalent to the amount of the iron group metals present. The following method is intended for such instances and aims at placing the analysis of phosphates on a level with ordinary analysis in both accuracy and simplicity.

Smith[1] found that a qualitative separation of the iron group metals in presence of excess of phosphate is possible at *p*H 3. Quantitatively, the optimum *p*H has been found to be 3·2–3·4. Haring and Leatherman[2] have shown that cobalt sulphide can be quantitatively precipitated at *p*H 4. Haring and Westfall[3] have shown that nickel sulphide is precipitated at *p*H 4·5. Similarly, Fales and Ware[4] have shown that zinc sulphide is completely precipitated at *p*H 2·0 and above. For the rapid precipitation of all three sulphides, *p*H 4·6–4·8 has been found best. The remaining principles of this new method are frequently employed in analysis.

ADVANTAGES OF THE METHOD.—Phosphate, instead of being removed, is employed to advantage. The adsorption losses and additional filtrations inevitably accompanying the removal of the phosphates are thus avoided. The method can be applied with facility when the phosphate is present in large excess over the iron and aluminium, under which conditions the classical methods fail.[5]

The metals barium and strontium, which are especially liable to be carried down by the iron group precipitate when phosphate is present, are removed first. The risk of losing these metals, owing to sulphate being present in the reagents used, is thus avoided and their detection is aided. There is no tendency for precipitates of a colloidal nature to be formed; zinc sulphide and calcium oxalate are often troublesome when precipitated from ammoniacal solutions. The iron

group phosphates can be filtered off much more readily than the basic acetates. Very selective reactions are employed and all the chemicals used are common and obtainable very pure. Adsorption errors compare very favourably with those in the classical methods of analysis.

PRINCIPLES OF THE METHOD.—(1) Groups 1 and 2 are removed as usual.

(2) Barium and strontium are precipitated as sulphates from a solution approx. $N/5$ in hydrochloric acid and of about 25 ml. in volume.

(3) Iron, aluminium and chromium are precipitated as phosphates at *p*H 3·2 to 3·4.

(4) The solution is then buffered to *p*H 4·6–4·8 and treated with hydrogen sulphide at 70° to 80° C. Zinc, nickel and cobalt sulphides are precipitated.

(5) After most of the hydrogen sulphide has been boiled off the manganese is precipitated as dioxide by oxidising with sodium hypochlorite.

(6) The calcium is precipitated as oxalate.

(7) The solution is finally made ammoniacal and magnesium is precipitated as phosphate.

(8) No provision is made for the detection of sodium and potassium, which must be tested for separately; this is more convenient and reliable, because a direct method minimises contamination with sodium and potassium from glassware and reagents and does not introduce large quantities of ammonium salts.

REAGENTS.—The following reagents are required:

5 *N* hydrochloric acid (dilute the concentrated acid to sp.gr. 1·084).

2 *N* sodium hydroxide solution.

M potassium dihydrogen phosphate solution (13·7 g. per 100 ml.).

3 *N* and *N* sodium acetate solutions.

M citric acid (21 g. per 100 ml.).

Sodium hypochlorite solution (commercial solution diluted to contain about 5 per cent. of available chlorine).

Bromophenol blue (0·1 g. of solid ground in a mortar with 4·1 ml. of $N/20$ sodium hydroxide solution and diluted to 250 ml.

$N/20$ sulphuric acid; $N/10$ and $N/100$ acetic acid; $N/10$ nitric acid.

3 *N* sodium nitrite solution (20 g. per 100 ml.).

A New Group Separation for the Qualitative and Quantitative Analysis of Phosphates

To cold soln. of 0·5 g. in 20 ml. add 2 *N* NaOH, 2–3 drops at a time and with stirring until a slight ppt. persists without appreciably dissolving in 20 secs. Add 1 ml. of 5 *N* HCl. Heat to incipient boiling, add 2 ml. of 10 per cent. Na_2SO_4 and, if there is a ppt. add another 2 ml. of Na_2SO_4 and heat in a boiling water-bath for 15 mins. Filter and wash with *N*/20 H_2SO_4.

Ppt. $SrSO_4$ $BaSO_4$

Try flame test and/or boil with 50 ml. of *N* Na_2CO_3. Decant through filter. Repeat process. Dissolve carbonates in acetic acid. Add K_2CrO_4: ppt. = $BaCrO_4$. To soln. add H_2SO_4: ppt. = $SrSO_4$.

Solution. Dilute to 100 ml. and while heating add 3 ml. of glacial acetic acid and then 2 ml. of 3 *N* sodium nitrite. Boil ½ min. Add 0–4 ml. of *M* KH_2PO_4 to ensure enough phosphate present to form Fe, Al and Cr phosphates. Add 3 ml. of *N* sodium acetate. Then try spot test with bromophenol blue and, if a yellow colour is obtained, add 1 ml. portions of sodium acetate soln. until a grey-green colour is obtained (pH 3·2 to 3·4). For quantitative analysis spot tests must now be washed back into the soln. Finally boil for a few secs. (3 mins. if Cr is present). Filter through a fast paper and wash ppt. preferably with hot *N*/100 acetic acid.

Ppt. $FePO_4$ $AlPO_4$ $CrPO_4$

Suspend in water, add H_2O_2 and NaOH. Boil, dilute and filter, $Fe(OH)_3$ remains. Acidify soln. with acetic acid, $AlPO_4$ is pptd. If soln. is yellow Cr is present.

Solution. Heat to boiling and ignore any ppt.; add 15 ml. of 3 *N* sodium acetate soln. Pass a fairly rapid stream of H_2S through the hot soln., preferably at 70° to 80° C. Absence of a ppt. in 2 mins. shows absence of Zn, Co or Ni; otherwise continue passage of gas for 5 to 10 mins. Heat nearly to boiling, filter and wash with *N*/10 acetic acid.

Ppt. ZnS CoS NiS

If Zn is present, white ZnS ppts. before the black CoS or NiS.

Solution (150–250 ml.). Boil off H_2S. To boiling soln. add NaOCl soln. at rate of about 1 drop per sec. Absence of a brownish ppt. with 2 ml. shows absence of Mn; otherwise continue treatment until ppt. darkens. Filter and wash ppt. once or twice with water, then with *N*/10 HNO_3, and finally with water.

Ppt. MnO_2

To confirm, extract with KHC_2O_4, deep red soln. proves Mn.

Solution. To hot soln. add 5 ml. of *M* citric acid and 20 ml. of sat'd. ammonium oxalate soln. After standing at least 5 mins. filter and wash with hot water, preferably containing a few drops of ammonium oxalate.

Ppt. $CaC_2O_4.H_2O$

Solution. Concentrate to about 150 ml., add 5 ml. of KH_2PO_4, and to hot soln. add a slight excess of NH_3, stir for a few mins. and finally add 20 ml. of conc. NH_3. Stand overnight. Filter and wash with 2 per cent. NH_3.

Ppt.	Solution.
$MgNH_4PO_4.6H_2O$	Reject.

Notes on the Procedure.—(1) *Preparation of the Solution.*—The quantity of metals present in the test solution should not be equivalent to more than 0·5 g. of the phosphates, so that if the sample is a metal or is mostly oxides it is advisable to take less of it. The 0·5 g. limit does not include Group 1 and 2 and alkali metals.

Organic substances or much ammonium salt must be absent. Under the conditions employed, ammonium salts prevent the oxidation of Mn to the dioxide;

in the absence of Mn ammonium salts do no harm and may be used in place of the sodium salts specified.

The solution of the substance should preferably be in dilute HCl and contain no metals of Group 1 or 2. Such a solution is obtained after Group 2 treatment, but would probably need concentrating to reduce to 20 ml.; this is essential only when strontium is present. If the solution is very strongly acid it is advisable to remove the excess acid by evaporation. Chromates are best first reduced, but provision is made in the scheme for this as well as for the oxidation of iron.

(2) *Precipitation of Barium and Strontium Sulphates.*—If sulphate is present in the test solution, and therefore barium and strontium are known to be absent, treatment with sodium sulphate may be omitted, and it is possible to proceed directly to the iron group separation.

The barium and strontium sulphates adsorb a small proportion of any ferric iron present, but, as at least 90 per cent. is left in solution, the adsorption of iron may be neglected for qualitative analysis. In quantitative analysis, when much iron is present together with barium or strontium, the adsorption of iron may be made negligible by reducing to the ferrous state as follows:

To the boiling solution in a conical flask add 1-ml. portions of 0·5 *N* hydriodic acid or sodium iodide until no more iodine is liberated; boil down to 20 ml. and proceed. Potassium iodide should not be used, because barium sulphate adsorbs potassium salts, giving high results. It is best to use a 100-ml. beaker when precipitating the sulphates.

If strontium is to be determined, heating in the water-bath should be continued for 30 minutes (or the solution left overnight), but for qualitative analysis 10 to 15 minutes are sufficient. When the substance contains a large proportion of calcium an excess of more than 0·2 g. of sodium sulphate should be avoided, but otherwise the specified 4 ml. of the solution may be used. When much calcium is present, the solution is best maintained at 25 ml. during the heating in the bath, and the sulphates should be washed until the washings give no reaction for calcium. Results are somewhat low for strontium when calcium is present in great excess of the amount of strontium. Traces of strontium that escape precipitation as sulphate are precipitated along with the calcium as oxalate.

(3) *Iron Group Phosphates.*—When it is known that the substance contains sufficient phosphate to form the iron group phosphates, it is undesirable to add more phosphate. The adsorption of metals and of phosphoric anhydride increases with the amount of excess phosphate. The adsorption of metals increases with rise in *p*H, but the adsorption of phosphoric anhydride decreases slightly. Dilution does not reduce the adsorption of zinc, but greatly reduces the adsorption of phosphoric anhydride.

If, after adding the 2 ml. of 3 *N* sodium nitrite solution and boiling, 1 ml. of *M* potassium dihydrogen phosphate solution is added and there is little or no precipitate, very little iron or aluminium can be present; in that event no more potassium dihydrogen phosphate solution need be added. If the solution is very green, indicating the presence of much chromium, or if in doubt, add 4 ml. of potassium dihydrogen phosphate solution. The *p*H at this stage is 2–2·5.

The nitrite assists in obtaining the phosphates in a good condition for filtration,

ensures oxidation of the iron and reduction of chromates, and will decompose the excess of iodide that may have been used to reduce iron; in that event, after addition of the nitrite, boiling should be continued until all the iodine is expelled. It may be necessary to stir the solution while heating, in order to prevent bumping.

Except when chromium or zinc is present, a single precipitation will usually suffice for quantitative analysis. When chromium and zinc are both present a single precipitation suffices for qualitative tests.

When re-precipitation is necessary, the phosphates should be dissolved in the minimum quantity of hydrochloric acid, 0·5 ml. of *M* potassium dihydrogen phosphate solution and 3 ml. of glacial acetic acid added, and the solution diluted to approx. 100 ml., heated to boiling, buffered to *p*H 3·2–3·4 by adding alkali acetate, boiled and filtered. The filtrate should be evaporated and most of the acetic acid expelled before adding it to the main filtrate. Throughout this method it is best to avoid boiling the solution in order to concentrate; it is preferable to evaporate the washings or second filtrate separately before adding them to the main solution.

Except when the solution is highly coloured, the buffering to *p*H 3·2–3·4 can be done with the bromophenol blue as internal indicator; the colour will change from yellow to greyish-green. A spotting method is always applicable and is more accurate, and a spot test should always be made to make certain of the *p*H. If there is any doubt about the correct colour, buffers of *p*H 3·0, 3·2 and 3·4 should be prepared by taking 3 ml. of acetic acid and 1, 2 and 4 ml. of *N* sodium acetate solution, respectively, and diluting to 100 ml. in each instance. These solutions should be used for spot tests; buffering will then cause no difficulty.

The solubility of iron phosphate rises very rapidly below *p*H 2·0 and that of aluminium phosphate below 3·0; when large amounts of both phosphate and zinc are present zinc phosphate begins to separate from boiling solutions at *p*H 3·6, nickel, cobalt and manganese at rather higher *p*H values, and calcium phosphate at 4·5. At *p*H 3·2–3·4 there are left dissolved 0·03 to 0·01 mg. of iron and 0·05 to 0·02 mg. of aluminium. A large excess of phosphate does not reduce the solubility of the iron and aluminium phosphates; *p*H is the primary factor.

Determination of Iron and Aluminium as Phosphates.—There is a tendency for too high results to be obtained owing to adsorbed P_2O_5, but a slight excess of P_2O_5 is necessary to prevent hydrolysis, otherwise low results are obtained. By adding the phosphate at the stage specified, particularly if the potassium dihydrogen phosphate is added slowly, the adsorption of P_2O_5 is greatly reduced. This is because, after the iron and aluminium phosphates have been precipitated, they adsorb little of the P_2O_5 subsequently added and much less than if all the phosphate has been added before precipitation occurred. Providing adsorption of metals is negligible, fair results can be obtained by weighing amounts up to 50 mg. of iron and aluminium phosphates; also larger amounts, provided that an excess of P_2O_5 was not originally present and the potassium dihydrogen phosphate is added slowly. Results are then usually not more than 3 per cent. high, and can be greatly improved by diluting to 200 ml. instead of 100 ml., as specified in the table.

Results correct to within 1 per cent. for amounts of 0·1 to 0·5 g. of iron and aluminium phosphates, and correct to within 1 mg. for amounts less than 0·1 g., can be obtained by re-precipitating as already described; at the same time adsorption of metals is reduced. The phosphates are washed with hot $N/100$ acetic acid until the washings give practically no reaction with silver nitrate, dried and ignited, gently at first, finally at bright red heat, until constant in weight. Aluminium phosphate, being very hygroscopic, must be weighed rapidly. A mixture of iron and aluminium phosphates can be dissolved in hydrochloric acid, iron determined volumetrically, and the aluminium obtained by difference.

All the alkali salts are removed by washing until free from chlorine. Washing with ammonium acetate or nitrate solution tends to give low results, owing to hydrolysis, whilst washing with ammonium phosphate solution tends to give high results owing to adsorption of P_2O_5. Ignition with filter-paper tends to reduce the phosphates, and for this reason filter-paper pulp as an aid to filtration is not advisable when the phosphates are to be weighed. Filtration is facilitated by keeping the funnel in a hot-water jacket or by placing it in a hot air-bath.

Chromium.—By this method all but about 2 mg. of chromium is precipitated when iron and aluminium are absent, but in their presence not more than 0·5 mg. of chromium remains in solution, and amounts of less than 2 mg. are almost completely carried down by 10 mg. of aluminium or iron phosphate. Traces of chromium remaining in solution do not interfere with subsequent separations and can be recovered after the magnesium test by boiling with tannic acid at *p*H 6–7.

Failure of chromium to be precipitated from acetate solutions appears to be due to complexes formed with the chloride, acetate and other ions (see Ref. 6). The stability of such complexes apparently increases with rise in *p*H from 2 to 7, for above *p*H 3 the precipitation of chromium phosphate becomes more and more sluggish, and only at *p*H 2·5 to 3·5 is precipitation in presence of chloride and absence of iron and aluminium, reasonably complete. In absence of iron and aluminium, the precipitation of chromium basic acetate fails at any *p*H.

The method can be used qualitatively when chromium is present and aluminium absent, but satisfactory quantitative separations can only be obtained when the chromium is present together with at least as much aluminium or iron, otherwise chromium is incompletely precipitated. Adsorption of zinc by chromic hydroxide is even greater than by chromium phosphate, being (by Blum's method) 57 per cent. as against 26 per cent. by this method. Zinc is usually best separated by a direct method.[4]

(4) *Zinc Group Sulphides.*—No difficulty should be experienced with this group, provided that a fairly rapid stream of hydrogen sulphide is used, so as to get the solution saturated. Two minutes' passage of the gas (70° to 80° C.) at the rate of 500 ml. per minute with the use of the usual glass tube, will completely precipitate 1 mg. of cobalt, whereas a very slow stream may fail even after 30 minutes. Precipitation occurs in the order: ZnS, CoS, NiS; hence when zinc is present together with nickel or cobalt, the white zinc sulphide can be seen before the black sulphides begin to be precipitated.

If the passage of gas begins at 90° to 100° C., and the beaker is allowed to stand on a paper mat, the solution generally remains at 70° to 80° C. long enough

for the precipitation of the sulphides to be completed. Temperature is not very important when only zinc sulphide is to be precipitated; at 70° to 80° C. not more than 0·5 mg. of nickel or cobalt remains dissolved at *p*H 4·6 to 4·8; if the solution saturated with hydrogen sulphide is boiled for a few seconds before filtering, not more than 0·02 mg. of nickel or cobalt remains in solution.

The *p*H of the solution during precipitation of the zinc group is 4·6 to 4·8, and the solution should give a blue colour with bromocresol green. Phosphates of zinc, cobalt, nickel and manganese may separate from the hot solution at this *p*H, but can be disregarded; the first three are decomposed by the hydrogen sulphide treatment, and manganese phosphate is readily dissolved by the *N*/10 acetic acid wash solution. When much manganese is present it is advisable in quantitative analysis to wash until a few drops of washings give no reaction for manganese with persulphate. Under these conditions the sulphides retain only a negligible amount of manganese.

(5) *Manganese.*—The precipitate obtained is so characteristic as to make confirmation redundant; the red colour obtained by dissolving the manganese dioxide in potassium acid oxalate solution is a specific, sensitive and easily applied test. The separation of manganese alone is very useful; different amounts from 0·2 g. of manganese to fractions of 1 mg. have been successfully separated from unknown substances and confirmed by direct methods of determining manganese.

The hypochlorite should not be added too quickly, or traces of manganese may be oxidised to a soluble red compound which is only decomposed by reducing. Should a red filtrate be obtained, a few drops of nitrite should be added and the liquid boiled, when the red colour will disappear; for rough qualitative analysis the red colour can be disregarded. For quantitative work the filtrate should always be treated a second time with hypochlorite to ensure completeness of precipitation. During precipitation of the manganese dioxide there is usually a sudden darkening almost to black, and if the hypochlorite has been added slowly (one drop per second) and the addition then stopped, there is no red colour and precipitation is generally complete.

The *N*/10 nitric acid wash solution serves to dissolve any calcium phosphate that may be precipitated by the prolonged boiling; after being washed in this way the manganese dioxide contains at most traces of calcium or magnesium.

The manganese dioxide may be dissolved in hydrochloric acid or oxalic acid and determined as phosphate, or small amounts may be dissolved in dilute sulphuric acid with the aid of sodium nitrite or sulphite and estimated colorimetrically.

Other reagents, such as bromine and persulphates, have been tried in place of hypochlorite, but are not so satisfactory, precipitation being slower and often incomplete with larger amounts of manganese. This method of precipitating manganese avoids the use of large amounts of reducing substances, such as formates, and ammonium salts, as reagents.

(6) *Calcium.*—The amount of magnesium adsorbed by the calcium does not usually warrant re-precipitation of the calcium oxalate. The acid solution containing magnesium and oxalate, unlike an ammoniacal solution, has no tendency to deposit magnesium oxalate.

If calcium is to be determined, the precipitate is best collected in a Gooch

crucible, dried and ignited (not above 600° C.), carbonated by moistening with saturated ammonium carbonate, dried and re-ignited for 3 minutes at 500° to 600° C., and weighed as calcium carbonate. Results are very satisfactory, no sodium salts being retained by the precipitate.

(7) *Magnesium.*—The precipitate may be filtered off, preferably in a Gooch crucible, and, after thorough washing with 2 per cent. ammonia, treated with a few drops of strong ammonium nitrate solution, dried, ignited and weighed as $Mg_2P_2O_7$. Results are very satisfactory.

TABLE I

ADSORPTION OF METALS BY THE IRON-GROUP PHOSPHATES

Taken g.	Adsorption Per Cent. (on added metal)	Taken g.	Adsorption Per Cent. (on added metal)
Cr ≡ 0·250 of $CrPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5*	26	Cr ≡ 0·250 of $CrPO_4$ Co ≡ 0·250 of $Co_3(PO_4)_2$ 0·355 of P_2O_5	5·5
Cr ≡ 0·250 of $CrPO_4$ Zn ≡ 0·025 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	52	Cr ≡ 0·250 of $CrPO_4$ Ni ≡ 0·250 of $Ni_3(PO_4)_2$ 0·355 of P_2O_5	3·5
Cr ≡ 0·250 of $CrPO_4$ Zn ≡ 0·005 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	55	Cr ≡ 0·250 of $CrPO_4$ Ca ≡ 0·250 of $Ca_3(PO_4)_2$ 0·355 of P_2O_5	5·0
Cr ≡ 0·250 of $CrPO_4$ Zn ≡ 0·001 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	50–60	Cr ≡ 0·500 of $CrPO_4$ Ca ≡ 0·025 of $Ca_3(PO_4)_2$ 0·355 of P_2O_5	12
Cr ≡ 0·100 of $CrPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·200 of P_2O_5	13	Cr ≡ 0·250 of $CrPO_4$ Mg ≡ 0·250 of $Mg_3(PO_4)_2$ 0·355 of P_2O_5	1·5
Cr ≡ 0·050 of $CrPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·142 of P_2O_5	7·5	Cr ≡ 0·10 of $CrPO_4$ Fe ≡ 0·10 of $FePO_4$ Al ≡ 0·10 of $AlPO_4$ Zn ≡ 0·16 of $Zn_3(PO_4)_2$ 0·284 of P_2O_5	14
Cr ≡ 0·250 of $CrPO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	8		
Cr ≡ 0·500 of $CrPO_4$ Mn ≡ 0·025 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	13	Cr ≡ 0·125 of $CrPO_4$ Al ≡ 0·125 of $AlPO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	3·4
Cr ≡ 0·050 of $CrPO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ 0·142 of P_2O_5	1·7	Cr ≡ 0·125 of $CrPO_4$ Fe ≡ 0·125 of $FePO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	7

* In this and the subsequent tables P_2O_5 represents the total amount of phosphate taken in each of the tests. It does not correspond to the amount of chromium and zinc phosphates together, but is merely a suitable quantity in excess of the amount of the metal or metals of the iron group used in the experiment.

Tables I, II and III show percentage adsorptions (calculated on the added metal) by the iron-group phosphates. From the work of Lundell and Knowles[5] it is evident that under the best conditions the ammonia method, phosphate being absent, gives results rather better for manganese, about the same for nickel and far worse for cobalt than this method. Under the same conditions (Blum's method, avoiding excess of ammonia) adsorption of zinc by iron is greater than by this new method; if excess of ammonia is added, adsorption of zinc is far less, but then the adsorption of nickel, cobalt and manganese becomes greater.

TABLE II

ADSORPTION OF METALS BY THE IRON-GROUP PHOSPHATES

Taken g.	Adsorption Per Cent. (on added metal)	Taken g.	Adsorption Per Cent. (on added metal)
Al ≡ 0·250 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·178 of P_2O_5	(at *p*H 3·2) 5	Al ≡ 0·050 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·142 of P_2O_5	1·5
Al ≡ 0·250 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	(at *p*H 3·2) 7 (at *p*H 3·4) 9	Al ≡ 0·250 of $AlPO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	1·2
Al ≡ 0·250 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·710 of P_2O_5	(at *p*H 3·2) 11	Al ≡ 0·500 of $AlPO_4$ Mn ≡ 0·025 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	4
Al ≡ 0·250 of $AlPO_4$ Zn ≡ 0·100 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	(at *p*H 3·2) 11	Al ≡ 0·250 of $AlPO_4$ Co ≡ 0·250 of $Co_3(PO_4)_2$ 0·355 of P_2O_5	1
Al ≡ 0·500 of $AlPO_4$ Zn ≡ 0·025 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	25	Al ≡ 0·250 of $AlPO_4$ Ni ≡ 0·250 of $Ni_3(PO_4)_2$ 0·355 of P_2O_5	0·5
Al ≡ 0·500 of $AlPO_4$ Zn ≡ 0·001 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	20–30	Al ≡ 0·250 of $AlPO_4$ Ca ≡ 0·250 of $Ca_3(PO_4)_2$ 0·355 of P_2O_5	<0·2
Al ≡ 0·100 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·200 of P_2O_5	3	Al ≡ 0·250 of $AlPO_4$ Mg ≡ 0·250 of $Mg_3(PO_4)_2$ 0·355 of P_2O_5	<0·2

Table IV shows that this method compares well with the basic-acetate method as regards adsorption. The great increase in adsorption of zinc with increase in *p*H by both the basic-acetate and the new method should be noted. The results shown for the basic-acetate method are probably better than those normally obtained, because usually the *p*H attained is higher—about 5·3; also more phosphate than will combine with the iron-group metals is often present, causing phosphates of subsequent groups to separate with the iron-group phosphates. The addition of ferric chloride after these have been precipitated does very little

good; sufficient iron-group metals to combine with all the P_2O_5 must be present at the time of buffering; alternatively, the solution should be buffered to *p*H 3, not *p*H 5, ferric chloride then added to remove the excess of P_2O_5 and the solution then buffered to *p*H 5 to precipitate the excess iron as basic acetate. Smith[1] has shown how unreliable the usual basic-acetate method is for mixtures containing much phosphate.

TABLE III

ADSORPTION OF METALS BY THE IRON-GROUP PHOSPHATES*

Taken g.	Adsorption Per Cent. (on added metal)	Taken g.	Adsorption Per Cent. (on added metal)
Fe ≡ 0·250 of $FePO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	10	Fe ≡ 0·125 of $FePO_4$ Al ≡ 0·125 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	10
Fe ≡ 0·250 of $FePO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	3·0	Fe ≡ 0·500 of $FePO_4$ Zn ≡ 0·025 of $Zn_3(PO_4)_2$ 0·355 of P_2O_5	30
Fe ≡ 0·500 of $FePO_4$ Mn ≡ 0·025 of $Mn_3(PO_4)_2$ 0·355 of P_2O_5	6	Fe ≡ 0·250 of $FePO_4$ Ba ≡ 0·250 of $Ba_3(PO_4)_2$ 0·355 of P_2O_5	30
Fe ≡ 0·250 of $FePO_4$ Co ≡ 0·250 of $Co_3(PO_4)_2$ 0·355 of P_2O_5	0·7	Al ≡ 0·250 of $AlPO_4$ Ba ≡ 0·250 of $Ba_3(PO_4)_2$ 0·355 of P_2O_5	25
Fe ≡ 0·250 of $FePO_4$ Ni ≡ 0·250 of $Ni_3(PO_4)_2$ 0·355 of P_2O_5	0·3	Cr ≡ 0·250 of $CrPO_4$ Ba ≡ 0·250 of $Ba_3(PO_4)_2$ 0·355 of P_2O_5	52
Fe ≡ 0·250 of $FePO_4$ Ca ≡ 0·250 of $Ca_3(PO_4)_2$ 0·355 of P_2O_5	<0·2	Cr ≡ 0·500 of $CrPO_4$ Ba ≡ 0·025 of $Ba_3(PO_4)_2$ 0·355 of P_2O_5	84
Fe ≡ 0·250 of $FePO_4$ Mg ≡ 0·250 of $Mg_3(PO_4)_2$ 0·355 of P_2O_5	<0·2	Cr ≡ 0·250 of $CrPO_4$ Sr ≡ 0·250 of $Sr_3(PO_4)_2$ 0·355 of P_2O_5	12

* The last five sets of results show what would happen if barium and strontium were not first removed but were precipitated with the calcium.

Table V demonstrates the adsorption effects throughout the various groups in the new phosphate separation method.

I gratefully acknowledge my indebtedness to Mr. H. C. S. de Whalley for testing out this method at the Tate and Lyle Research Laboratory; also to Dr. Harold Toms of East Ham Technical College, where much of the practical work was done.

TABLE IV

ADSORPTION OF BIVALENT METALS BY THE BASIC-ACETATE METHOD WITH PHOSPHATE PRESENT*

Taken g.	*p*H by bromo-cresol green	Adsorbed by the basic-acetate precipitate	Adsorbed by new method Per Cent. (on added metal)
Fe ≡ 0·250 of $FePO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $FePO_4$	4·8	13·5 per cent. of the zinc	10
Fe ≡ 0·250 of $FePO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $FePO_4$	4·8	4·5 per cent. of the manganese	3·0
Fe ≡ 0·250 of $FePO_4$ Ni ≡ 0·250 of $Ni_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $FePO_4$	5·0	6 per cent. of the nickel	0·3
Fe ≡ 0·250 of $FePO_4$ Ba ≡ 0·250 of $Ba_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $FePO_4$	5·0	14 per cent. of the barium	none
Al ≡ 0·250 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $AlPO_4$	4·4	6 per cent. of the zinc	8
Al ≡ 0·250 of $AlPO_4$ Zn ≡ 0·250 of $Zn_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $AlPO_4$	4·8	15 per cent. of the zinc	8
Al ≡ 0·250 of $AlPO_4$ Mn ≡ 0·250 of $Mn_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $AlPO_4$	4·4	0·5 per cent. of the manganese	1·2
Al ≡ 0·250 of $AlPO_4$ Ni ≡ 0·250 of $Ni_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $AlPO_4$	4·6	1·3 per cent. of the nickel	0·5
Fe ≡ 0·10 of $FePO_4$ Al ≡ 0·10 of $AlPO_4$ Cr ≡ 0·10 of $CrPO_4$ P_2O_5 ≡ ½ of the Fe, Al and Cr	4·3	20 per cent. of the zinc	14
Fe ≡ 0·250 of $FePO_4$ Co ≡ 0·250 of $Co_3(PO_4)_2$ P_2O_5 ≡ 0·125 of $FePO_4$	4·7	4·0 per cent. of the cobalt	0·7

* The method employed for these basic-acetate separations was that of Treadwell and Hall, Vol. II, 1930; 1·0 to 1·2 g. of crystalline sodium acetate was used.

TABLE V

ADSORPTION EFFECTS THROUGHOUT THE GROUPS IN THE NEW PHOSPHATE SEPARATION METHOD

Taken g.	Adsorption, etc.
Zn ≡ 0·200 of ZnS Mn ≡ 0·200 of MnS 0·142 of P_2O_5	1·4 per cent. of the manganese carried down by the zinc sulphide.
Ni ≡ 0·200 of NiS Mn ≡ 0·200 of MnS 0·142 of P_2O_5	0·5 per cent. of the manganese carried down by the nickel sulphide. Not more than 0·25 mg. of nickel remained in the filtrate from the nickel sulphide.
Co ≡ 0·200 of CoS Mn ≡ 0·200 of MnS 0·142 of P_2O_5	0·3 per cent. of the manganese carried down by the cobalt sulphide. Not more than 0·25 mg. cobalt remained in the filtrate from the cobalt sulphide.
Mn ≡ 0·200 of MnO Ca ≡ 0·200 of CaO 0·142 of P_2O_5	Not more than 0·5 per cent. of the calcium retained by the manganese dioxide. Obtained Ca ≡ 0·1986 g. of CaO. ,, Mn ≡ 0·2017 g. of MnO.
Zn ≡ 0·200 of ZnO Ca ≡ 0·200 of CaO 0·142 of P_2O_5	Not more than 0·2 per cent. of the calcium carried down by the zinc sulphide.
Ca ≡ 0·150 of CaO Mg ≡ 0·150 of MgO 0·142 of P_2O_5	1·5 per cent. of the magnesium carried down by the calcium oxalate. Obtained 0·1530 g. of CaO. ,, 0·1480 g. of MgO.
Ba ≡ 0·350 of $BaSO_4$ $Fe^{\cdots}$ ≡ 0·242 of Fe_2O_3 0·213 of P_2O_5	3 per cent. of the iron adsorbed by the barium sulphate.
Ba ≡ 0·250 of $BaSO_4$ $Fe^{\cdots}$ ≡ 0·250 of $FePO_4$ 0·100 of P_2O_5	After reduction of the iron by hydriodic acid 0·2 per cent. of the iron adsorbed by the barium sulphate. Obtained 0·2517 g. of $BaSO_4$.
Sr ≡ 0·151 of $SrSO_4$ $Fe^{\cdots}$ ≡ 0·242 of Fe_2O_3 0·213 of P_2O_5	1 per cent. of the iron adsorbed by the strontium sulphate. Filtrate contained not more than 1 mg. of strontium.
Sr ≡ 0·010 of SrO $Fe^{\cdots}$ ≡ 0·500 of $FePO_4$ 0·142 of P_2O_5	After reduction with hydriodic acid. $SrSO_4$ ≡ 0·0094 g. of SrO obtained containing 0·0002 g. of Fe_2O_3.
Sr ≡ 0·010 of SrO $Fe^{\cdots}$ ≡ 0·500 of $FePO_4$ 0·142 of P_2O_5	$SrSO_4$ ≡ 0·005 g. of SrO obtained.
Sr ≡ 0·050 of $SrSO_4$ Ca ≡ 0·500 of $CaSO_4$ 0·100 of P_2O_5	0·041 g. of $SrSO_4$ obtained.
Sr ≡ 0·250 of $SrSO_4$ 0·100 of P_2O_5	5 minutes' heating in bath gave 0·240 g. of $SrSO_4$. 15 ,, ,, ,, ,, ,, 0·245 g. ,, ,, 30 ,, ,, ,, ,, ,, 0·249 g. ,, ,,

REFERENCES

1. T. B. Smith, *J. Chem. Soc.*, 1933, 253.
2. M. E. Haring and M. Leatherman, *J. Amer. Chem. Soc.*, 1930, **52**, 5135.
3. M. E. Haring and B. B. Westfall, *id.*, 1930, **52**, 5141.
4. H. A. Fales and G. M. Ware, *id.*, 1919, **41**, 487; also their textbook, "*Inorganic Quantitative Analysis*," Bell, pp. 250–257 (1928).
5. G. E. F. Lundell and H. B. Knowles, *J. Amer. Chem. Soc.*, 1923, **45**, 676.
6. J. Mellor, "*Inorganic Chemistry*," Vol. XI, p. 374 (1931).

18, STRATTON DRIVE
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The Peroxide Accumulation Rate of Oils—Relationship to Mackey Test Results and Oxidation on Textiles

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THE work described below relates to esters (especially glycerides) of fatty acids, and some of the conclusions reached are not altogether applicable to fatty acids.

When oils (*e.g.* olive oil) are employed for fibre lubrication in wool-combing, 3 per cent. of oil is applied to the wool. The surface area of the fibres in 1 lb. of wool is approximately 70 square yards, and 1 lb. of oil is therefore spread over a maximum surface of about half-an-acre. The conditions are very favourable to rapid oxidation; this is necessary in order to produce fission products which are pale in colour and easily scoured out, but in certain circumstances it may result in the formation of resinous or paint-like polymers which are yellowish or brownish in colour and difficult to remove from the fibre by an alkaline soap scour. In some instances the oxidation (which is exothermic in character) may be so rapid as to cause spontaneous ignition of the oiled fibres. The suitability or otherwise of an oil for textile use cannot be predicted from the usual analytical data such as the iodine value, amount of unsaponifiable matter, etc. For textile and fire insurance purposes, it has been the custom to rely on the evidence furnished by the Mackey test.[1,2]

The four main factors influencing the rate of oxidation of a non-drying oil are: (*a*) the surface/mass ratio of the oil during the experiment; (*b*) the activity of oxidising catalysts present; (*c*) the activity of antioxidants present; (*d*) the temperature conditions.

The chemical constitution of the oil is of very minor importance,[3,4] compared with the catalyst-antioxidant balance. Temperature rise in the Mackey apparatus is probably due to heat liberated during peroxide formation and decomposition, both these reactions being accelerated by rise in temperature. For reasons to be discussed elsewhere,[5] the rate of accumulation of peroxides (*i.e.* formation rate minus decomposition rate) in an oil which is exposed to light and air should be a measure of the effective activity (*i.e.* catalyst activity minus antioxidant activity) of the oxidising catalyst content.

The peroxide-content *per se* of an oil has been proposed as a criterion of the safety of a textile oil, but this appears to us to be of no significance. A given peroxide-content may be due to slow accumulation over a long period, the effective catalyst activity being low; or to rapid accumulation over a short period, the effective catalyst activity being high; it is assumed that other influencing conditions (light and heat) are equal. Conversely, two oils may show the same degree of effective catalyst activity, but have different peroxide-contents due, for example, to difference in age.

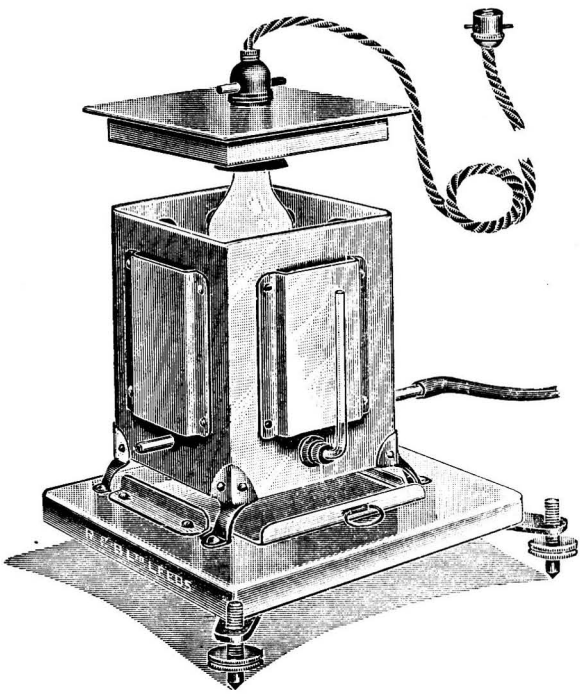

Fig. 1

The peroxide-content of an oil is a record of past history rather than an indication of future behaviour (see Table I), whilst the peroxide accumulation rate measures the liability of the oil sample to oxidise rapidly.

TABLE I

Oil	Description	Peroxides		Mackey test		
		P.I.V.	P.A.R.	°F at 5 hrs.	Mins. to reach 400°F.	Iodine value
Arachis	raw	6·1	54·6	—	95	92·4
,,	Nilox—processed ..	0·5	1·6	215	—	86·5
Olive	Syrian ,, 5 per cent.	5·7	37·4	—	120	84·0
,,	Nilox ,, ...	0·6	1·2	210	—	84·3
,,	Malaga salad	8·3	15·6	224	—	82·9
,,	African	56·9	51·3	—	113	84·1
,,	,, stored in dark 8 months to destroy peroxides ..	0·5	35·7	—	110	85·1

The apparatus shown in Fig. 1 (p. 348) was therefore devised to measure the peroxide accumulation rate (P.A.R.). It consists of a tray carrying four shallow glass dishes (3-inch square Petri dishes). Oil is placed in one of the dishes, which is then exposed to the light from a 100-watt electric lamp, the heat rays being reduced by means of a 2-inch layer of running cold water. Under these conditions peroxides are usually formed quite rapidly; the difference between the amount of peroxide present before and after exposure is a measure of the peroxide accumulation rate.

The details of the test are as follows:—The peroxides in 1 g. of the oil sample are determined by the method of Lea,[4] but calculated to give the number of mg. of iodine liberated per 1 g. of oil, this being termed the peroxide iodine value (P.I.V.). A peroxide iodine value of 1 indicates that roughly 1 mol. in 2000 is a peroxide (with ethyl oleate). Ten g. of the oil are then weighed into one of the Petri dishes and exposed to light in the apparatus for 48 hours. The P.I.V. is determined on the exposed sample, and the difference between exposed and unexposed value is termed the peroxide accumulation rate.

The variables in the test are:—(*a*) the four positions in the tray (See Table II); (*b*) the weight of oil per tray (*i.e.* surface/mass ratio) (See Table II); (*c*) the intensity of the light (See Table II); (*d*) the temperature of the oil (See Table II); (*e*) the method of determining peroxides; (*f*) time (See Table III); (*g*) oxidising catalyst activity (See Tables IV and V); (*h*) antioxidant activity (See Table V).

TABLE II

Experiment No.	Variations from standard conditions	Initial P.I.V.	P.A.R. of oil in position 1	2	3	4
1	None	1·2	16·6	17·4	19·2	17·6
2	60-Watt lamp instead of 100—Air current blown over dishes	1·2	8·2 —	7·9 —	— 8·9	— 8·5
3	Amount of oil in dish	1·2	18·4 (5 ml.)	17·2 (10 ml.)	15·2 (20 ml.)	11·8 (40 ml.)
4	Standing water; mean temp. 35° C.	1·2	20·8	17·5	19·5	18·2
5	No light	1·2	1·3	1·3	1·2	1·3

TABLE III

Hours	Iodine value	Fall in I.V. from previous reading	Peroxide iodine value	Rise in P.I.V. from previous reading
0	83·3	—	27·2	—
8	82·3	1·0	33·0	5·8
16	80·3	2·0	38·6	5·6
32	80·3	0·0	42·0	3·4
40	78·9	1·4	48·0	6·0
48	78·4	0·5	53·2	5·2
60	74·6	3·8	69·2	16·0
	Total	8·7		42·0

Note I. The 60-hour product heated *in vacuo* at 170° C. for 3 hours had I.V. **79·6**, and P.I.V. 20, showing that peroxide formation is to some extent reversible.

Note II. A fall of one unit in iodine value corresponds with an increase of **4·8** in P.I.V.; this relationship indicates $O_2=I$. The expected relationship is $O_2=I_2$.

TABLE IV

Oil	Ferric oleate added Per Cent.	Initial P.I.V.	P.A.R.
Ethyl oleate ..	0·0	2·0	8·0
	0·1	2·8	24·5
Olive oil	0·0	7·0	22·3
	0·1	7·4	66·5

TABLE V

Oil	Treatment or addition	Peroxides		Mackey test		Storage of oiled wool					
						Iodine value			Free fatty acid		
		Initial P.I.V.	P.A.R.	°F. at 5 hrs.	Mins. to 400°F.	Initial	After 7 days' normal storage, drop	Drop in ageing test	Initial	Increase after 7 days' normal storage	Increase in ageing test
Olive	None	2·2	28·1	—	—	84·8	4·4	22·3	3·0	1·2	3·4
,,	0·01% ferric oleate	2·1	56·7	—	—	84·8	—	25·0	3·0	1·2	4·4
,,	0·25 per cent. β-naphthol ..	2·3	10·6	—	—	85·0	5·2	19·1	3·2	0·2	2·1
,,	Nilox-processed ..	2·2	0·4	—	—	84·4	4·5	11·0	3·1	0·1	1·3
Olive	None	7·04	33·3	—	109	85·4	—	31·7	3·0	—	6·1
,,	Nilox-processed	1·8	4·2	234	—	84·8	—	15·6	3·1	—	1·3
Arachis	None	4·8	51·8	—	65	92·7	—	54·2	3·8	—	7·4
,,	Nilox-processed[7,8]	0·2	3·2	210	—	87·3	—	11·8	0·1	—	2·2
Ester Oil S[9]	Nilox-processed ..	8·9	4·1	222	—	42·7	—	10·0	3·1	—	2·6

Variables (*a*), (*b*) and (*e*) can be controlled exactly, and variations in (*d*) under normal laboratory conditions do not affect the results; variations in (*c*) are likely to be serious (owing to such causes as voltage drops in the mains supply, ageing of the bulb, deposits in the water-tray); hence a control test on a standard oil should always be included.

It should be pointed out that most antioxidants of the amine or phenol types are liable themselves to develop coloured oxidation products, and this may result in the production of stains on textile fibres. Moreover, once the effectiveness of an antioxidant is exhausted, the oil will become oxidised according to its nature; *e.g.* cotton seed or linseed oils may be stabilised by β-naphthol to give an excellent P.A.R. or Mackey test, but would yet be very unsuitable for textile purposes; this applies also to an olive oil containing considerable amounts of catalysts, but stabilised by additions of antioxidant. Antioxidants merely delay the onset of the oxidation, but do not affect the type of end-product; an oil yields the same type of oxidation product when antioxidant is present (though more slowly) as when it is absent.[10]

Table V indicates the parallel relationship between the P.A.R., the Mackey test, and the rate of oxidation when oiled wool sliver holding 3 per cent. of oil was (*a*) subjected to air at room temperature for 7 days, and (*b*) subjected to an "ageing test" (consisting of storing for seven days in a moist atmosphere at 90° C.). It is clear that there is qualitative agreement between the P.A.R., Mackey test, normal oxidation and ageing test.

SUMMARY.—(1) The oxidation of an oil should be considered from two aspects which are almost completely unrelated, *viz.*: (*a*) the *rate of oxidation*, which depends upon the catalyst/antioxidant balance; (*b*) the *type of end product*, which depends upon the amount of di- and poly-ethenoids present.

(2) The P.A.R. indicates clearly the existing catalyst—antioxidant balance: large P.A.R. figures go with bad Mackey tests, and rapid oxidation in use on textiles.

(3) The P.A.R. does not give any reliable indication of: (*a*) the amount of oxidising catalysts, as catalyst activity may be masked by antioxidants; (*b*) the type of end-product of oxidation (this may be examined by means of the alkaline ageing test[10]).

(4) A high P.A.R. always indicates an oil unsuitable for textile purposes, but a low P.A.R. does not necessarily indicate suitability, as very unsaturated oils can be prepared with low rates of oxidation.

(5) The P.A.R. seems to afford the same kind of information as the Mackey test and is easier to carry out consistently. Further, direct comparison can be made of two, three, or four oils under identical conditions. Both tests should be regarded as a means of condemning bad oils rather than of approving good ones. A good result may mean either: (*a*) the oil is free from oxidising catalysts, or (*b*) the catalyst activity has been swamped by antioxidants (an undesirable state of affairs).

(6) The P.I.V. of an oil bears no direct relationship to the iodine value, rate of oxidation in use, Mackey Test, P.A.R., or catalyst/antioxidant balance, and is almost useless as a means of evaluating textile oils.

REFERENCES

1. W. McD. Mackey, *J. Soc. Chem. Ind.*, 1896, **15**, 90.
2. W. Garner and W. Leach, ANALYST, 1936, **61**, 337.
3. W. Garner, *id.*, 1936, **61**, 519.
4. —— *J. Text. Inst.*, 1937, **28**, 57–68.
5. —— *J. Soc. Dyers Col.* (in press).
6. Lea, *Proc. Roy. Soc.*, 1931, 175.
7. *Wool Record*, 1939, April 6th.
8. Wool Industries Research Association Special Report on Nilox Arachis Oil.
9. Wool Industries Research Association Special Report on Ester Oil.
10. W. Garner, ANALYST, 1940, **65**, 219.

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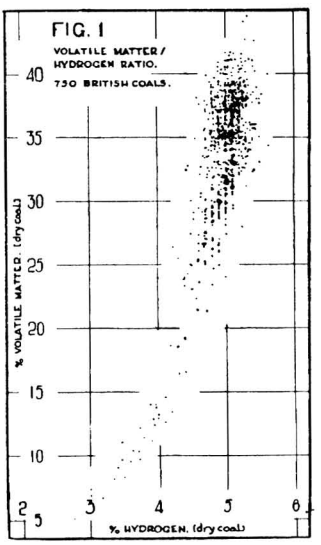
Notes

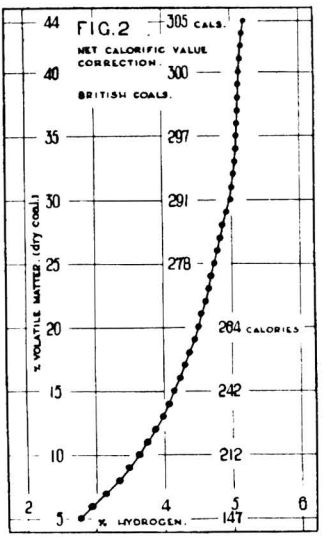
The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.

THE NET CALORIFIC VALUE OF COALS

THE analyses normally required to control the quality of coal consignments are the proximate analysis, the sulphur-content and the gross calorific value. Analysts are frequently asked to provide, in addition, the net calorific value. This requires a further analytical determination, that of the hydrogen-content of the coal.

The examination of some seven hundred and fifty analyses of representative British coals indicates that it is possible to calculate the net calorific value by the use of the data available in the proximate analysis and without the additional determination of the hydrogen-content.





The relationships between carbon, hydrogen, volatile matter and gross calorific value have been noted and examined in detail by Seyler.[1] Recently the same investigator[2] has shown that the four macro-petrological constituents of coal—vitrain, clarain, durain and fusain—are aggregations of one or more micro-petrological units or "macerals," analogous to the minerals of inorganic rock types. Seyler[3] has also been able to demonstrate, by the examination of selected coals, that the micro-petrological units—vitrinite, fusinite, exinite and resinite, differ in chemical character; for example, macerals of different type but of the same elementary analysis vary in volatile matter content.

Industrial supplies of coal are composed mainly of clarain and durain—macro-constituents which are themselves mixtures of varying amounts of the macerals, vitrinite, fusinite, exinite and resinite. The hydrogen-content of coal samples of the same volatile matter content will therefore vary within limits according to the types and the amounts of the macerals present in each sample.

The variation in the ratio of volatile matter to hydrogen-content at any one value for volatile matter content may be judged from the graph in Fig. 1, in which the hydrogen-content on the dry basis is plotted against volatile matter for some

750 British coals. The examination of this large number of analyses has been made possible by the publication in the Technical and Survey Papers of the Fuel Research Board of full analyses of representative seam and commercial coal samples from most British coalfields other than South Wales. The data for low volatile South Wales coals have been abstracted from analyses made at University College, Cardiff.

The average hydrogen-content at any one specific volatile matter content is given by the graph of Fig. 2, drawn as a smoothed curve based on the computed average hydrogen values at 1 per cent. volatile matter intervals. In Fig. 2 are also shown the average net calorific value corrections at stated volatile matter intervals. Fig. 2 may also be used conveniently when directly determined hydrogen values are available.

A study of the data used for the construction of Figs. 1 and 2 shows that over 50 per cent. of the values for hydrogen-content are within ±0·1 per cent. of the average value as read from Fig. 2, 78 per cent. within ±0·2 per cent., 93 per cent. within 0·3 per cent., and 99 per cent. within ±0·5 per cent. In terms of net calorific value corrections, 0·1 per cent., 0·3 per cent., and 0·5 per cent. hydrogen are respectively 5, 15 and 26 calories. If an experimental variation of the order of 25 calories is accepted as reasonable in the direct determination of the gross calorific value, then the graphical method of calculating the net calorific value by means of the volatile matter value is fully justified.

This method of calculation of the net calorific value may not be applicable to coals from overseas. In ninety analyses of representative American coals, published by Haslam,[4] the hydrogen percentage derived from the volatile matter by the use of Fig. 2 is consistently lower than the determined value, the difference being as high as 2·0 per cent. with some high volatile American bituminous coals. It may be that the perhydrous macerals, exinite and resinite, occur in greater quantity and with greater variation in typical coals of the U.S.A.

T. EVANS

REFERENCES

1. C. A. Seyler, *Proc. South Wales Institute of Engineers*, April, 1900.
2. —— *Fuel*, Jan., 1936.
3. —— *Proc. South Wales Institute of Engineers*, Jan., 1938.
4. R. T. Haslam and R. P. Russell, "*Fuels and their Combustion*," New York, 1926, p. 68.

DEPARTMENT OF METALLURGY AND FUEL TECHNOLOGY
UNIVERSITY COLLEGE, CARDIFF

May, 1940

Notes from the Reports of Public Analysts

The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports are submitted to the Publication Committee.

MUNICIPALITY OF BANGALORE

ANNUAL REPORT OF THE PUBLIC ANALYST FOR THE YEAR 1939–40

THE Bangalore Prevention of Adulteration Act, 1937, came into operation in October, 1938, and the analytical work is carried out in the Municipal Laboratory, Civil and Military Station, Bangalore. During the last three months of 1938 the total number of samples examined was 787, comprising 423 of milk, 284 of ghee, 90 of butter and 10 of tea. Of these samples, 57 per cent. were adulterated, the percentage for each class being milk 47, ghee 73, and butter 60. All the samples of tea were genuine.

The milk sold in the Station is derived from the buffalo and the cow, but the latter is preferred, as in other parts of the country. Adulteration of cows' milk with buffalo milk is therefore very prevalent, in addition to the usual admixture with water. A small percentage of the adulterated samples contained separated milk, and in one sample boiled and fermented milk (commonly known as buttermilk in Bangalore) was detected.

The adulterated samples of ghee and butter contained various amounts of arachis oil, sesame oil, coconut oil and foreign animal fats. In some samples the vegetable fats constituted nearly 95 per cent. of the mixtures.

G. NARASIMHA MURTY

Department of Scientific and Industrial Research

METHODS FOR THE DETECTION OF TOXIC GASES IN INDUSTRY

ARSINE*

OCCURRENCE.—Arsine (arsenic trihydride) occurs in potentially dangerous concentrations in many industries, notably the manufacture of zinc chloride and sulphate and the smelting of arsenical ores; also in electroplating and galvanising works and in the manufacture of dyestuffs and of hydrochloric and sulphuric acids.

POISONOUS EFFECTS.—There is nearly always some delay—sometimes a day or two—in the onset of symptoms. At first these are usually indefinite; there may be severe headache, giddiness, nausea and vomiting. In more severe cases the vomiting may be more pronounced, and the urine dark or stained with blood. After a day or two there is severe anaemia, and the skin becomes jaundiced.

Exposure for 1 hour to an atmosphere containing 1 part of arsine in 20,000 is dangerous, and exposure for 12 hours to a concentration of 1 in 100,000 may be fatal. There are no recorded figures as to the limits of concentration that may be regarded as harmless for continuous daily exposure, but there is evidence that repeated exposures to very low concentrations may have cumulative effects resulting in severe poisoning.

METHODS OF DETECTION.—Although silver nitrate test-paper is about 20 times as sensitive as mercuric chloride paper, it has the drawbacks that the stains vary in colour from yellow to black according to the temperature of drying; that they darken very rapidly, so that accurate matching is difficult, and that the papers do not keep well.

In the standard test adopted, the test-papers are made by immersing the strips in 5 per cent. mercuric chloride solution, drying them, and cutting off and discarding the ends.

The atmosphere under examination is drawn by means of a hand-pump of specified dimensions through the test-paper, and the resulting stain (if any) is compared within 5 minutes with the standard stains issued with the leaflet. The concentration of arsine is then found by reference to the colour chart, which shows the intensities of stains corresponding with 10 to 50 strokes of the pump. In this way concentrations of arsine down to 1 in 250,000 (0·013 mg. per litre) can be detected. The sample of the atmosphere is passed through lead acetate paper to absorb any traces of hydrogen sulphide before coming in contact with the mercuric chloride paper.

* Leaflet No. 9. H.M. Stationery Office. Price 2s. 6d. net. Further copies of the standard stains. Price 2s.; by post 2s. 3d.

FOOD INVESTIGATION

THE FUNCTION OF NITRATE, NITRITE AND BACTERIA IN THE CURING OF BACON AND HAMS*

THE method of manufacturing bacon by tank curing in this country has been described in detail by Callow (*Biochem. J.*, 1929, **23**, 648); it differs in many respects from the methods used in Canada and the United States, particularly in the temperature of curing. Also, in 1925, the use of sodium nitrite was authorised in the United States, provided that the amount in the finished product did not exceed 200 p.p.m. Sodium nitrite is also permissible in France, Germany, Holland, the Argentine, and other countries; in this country its use is illegal.†

The object of the present investigation was to determine the relative importance of the various factors contributing to the cured flavour of bacon, and also to ascertain if the use of sodium nitrite in the English method would give a satisfactory product (*cf.* Osman Jones, ANALYST, 1933, **58**, 140). The colorimetric method of Lewis and Blake (*Allen's Organic Analysis*, 5th Ed., Vol. IX, pp. 420, 432), in which the reagents consist of α-naphthylamine and sulphanilic acid in hydrochloric acid, was used for the determination of nitrite.

ACTION OF HEAT ON NITRITE IN BACON.—It was found that with initial concentrations of 30 to 589 p.p.m. of sodium nitrite the time required to destroy about half of the nitrite increased with the concentration, *e.g.* from 13 to 120 minutes. Hence the usual times of cooking are unlikely to reduce the nitrite-content from a high to a low value.

NITRITE AND FIXATION OF COLOUR.—In absence of oxygen nitroso-haemoglobin is stable in solution. This *residual* nitrite in the tissue appears unnecessary for the fixation of colour, although it may possibly help to preserve the colour of cut surfaces exposed to the air.

FORMATION OF NITRITE FROM NITRATE AFTER CURING.—Judging by the concentration of potassium nitrate in the pickle, lean bacon may contain up to about 0·5 per cent. of nitrate. In one experiment with sliced bacon stored at 10° C. the nitrite-content increased from 22 p.p.m. to 260 p.p.m.—520 p.p.m. after 9 days.

NITRITE, SODIUM CHLORIDE AND WATER IN COMMERCIAL BACON.—Forty samples of bacon of the best quality (including Canadian, Danish, English, Irish and Swedish) contained from 8 to 204 p.p.m. of nitrite (as sodium nitrite) with a mean value of 57. The sodium chloride ranged from 3·0 to 7·6 per cent.; mean, 5·5 per cent., and the water from 53·9 to 68·2; mean, 64·1 per cent.

NITRATE, NITRITE AND FLAVOUR.—Tests applied to 23 of the above-mentioned 40 samples of bacon showed no correlation between flavour and nitrite-content; bacon containing as little as 10 p.p.m. had a satisfactory flavour. So far as can be ascertained, the distinctive flavour of bacon and ham is due to a reaction between the nitrite and constituents of the tissue either during curing or cooking.

BACTERIAL PRODUCTS AND FLAVOUR.—Contrary to a view that is held, the tests indicated that a good bacon flavour is produced by the action of sodium chloride and nitrite on flesh constituents, and that neither the presence of nitrate nor the action of bacteria is essential.

BACTERIAL FLORA OF TANK-PICKLES AND BACON.—Commercial pickle gave a very high count of bacteria, almost entirely micrococci when grown on ordinary media. Nearly all reduced nitrate—some even in 25 per cent. solutions of sodium chloride. The number of micro-organisms isolated was about 10^6 per ml., of which

* Special Report No. 49. By J. Brooks, Ph.D., R. B. Haines, D.Sc., Ph.D., T. Moran, D.Sc., Ph.D., and J. Pace, Ph.D. H.M. Stationery Office, York House, Kingsway, London, W.C.2. April, 1940. Price 9d. net.

† The Minister of Health made Regulations on October 20th, 1939, and April 30th, 1940, whereby the addition of sodium or potassium nitrite to bacon or ham is now permissible (see ANALYST, 1939, **64**, 882; 1940, **65**, 359).

90 per cent. were viable only on special media. Commercial samples of bacon gave counts of 10^6 or more viable organisms per sq.cm. of superficial tissue; they were mainly micrococci or yeasts or both. It seems unlikely that the development of bacterial flora has any material influence on the flavour.

ADVANTAGES OF THE USE OF NITRITE.—The direct use of nitrite in curing would enable the nitrite concentration in the pickle to be more rigorously controlled. At the same time, other constituents (sodium chloride, nitrate, flavouring ingredients, etc.) could be varied as desired, and the *p*H could be controlled. The bacon would keep better owing to the reduction in the surface bacteria flora, and the temperature of curing could be reduced, since it would no longer be necessary to maintain a balanced microflora in the tank-pickle.

Institute of Brewing

REPORT OF BREWERY EFFLUENTS COMMITTEE*

IN 1938 the Turton (Lancashire) Urban District Council put forward, under the Public Health (Drainage of Premises) Act, 1937, draft bye-laws, in which it was proposed: (1) to limit the amount of solids in suspension in trade effluents to 20 grains per gallon; (2) to prohibit the presence of yeast; (3) to prohibit the presence of sugar. The Brewer's Society drew the attention of the Institute to these proposals, and suggested that an investigation should be made to ascertain reasonable limits for solids in suspension, yeast and sugar.

As the result of this request a Brewery Effluents Committee was formed, consisting of Messrs. Bernard M. Brown (*Chairman*), Julian L. Baker, F. P. Clift, Harold Heron, H. B. Hutchinson, H. R. Lyell, Sir Gilbert Morgan, and W. H. Bird (*Secretary*).

This Committee considered the evidence immediately available as to the constitution of brewery effluents, and also obtained the expert opinion of Dr. E. Ardern. In March, 1939, a Draft Report was issued asking for comments and pertinent information, and 40 replies were received. Only 4 gave recorded figures, and these fell within the ranges already considered.

CONSTITUTION OF BREWERY EFFLUENTS.—*Suspended Solids.*—Figures ranging from 5 to 1100 grains per gallon were shown by single samples. In one recently-constructed brewery averaged samples gave figures ranging from 6 to 67 grains per gallon, with a mean value of 32 grains per gallon.

Yeast.—In the above-mentioned brewery averaged 24-hour samples gave figures ranging from 265 to 8607 yeast cells per ml., with a mean value of 1774 cells; these figures are equivalent to 1·2, 37·7 and 7·8 grains of dry yeast per gallon. Single samples from another brewery gave figures ranging from *nil* to 260,000 yeast cells per ml., corresponding with *nil* to 823 grains of dry yeast per gallon.

Sugar.—Averaged samples from one brewery contained from 1·7 to 16·1 grains of reducing sugar (expressed as glucose) per gallon. At another brewery seven of eight single samples were free from sugar, and one contained sugar equivalent to 150 grains of glucose per gallon.

RECOMMENDATIONS.—(1) *General Considerations.*—It is pointed out that such bye-laws as may be proposed under the 1937 Act apply only to effluents from new premises, from premises to which an addition has been made, or from which on any one day a greater quantity of effluent is discharged into the public sewers than was discharged on any one day in the year ending March 3rd, 1937.

This consideration, however, is subject to the proviso that bye-laws which impose regulations regarding the temperature and neutralisation of trade effluents,

* *J. Institute Brewing*, 1939, **45**, 551–555.

and the provision of inspection chambers or manholes and meters will apply to effluents, even though the consent of the Local Authority is not required under the Act to the discharge of such effluents.

Before bye-laws can be applied to brewery effluents, it will be necessary, in order to obtain samples that will conform to any reasonable standard, either to bring all outlets from the premises to one point commanding the public sewer, where a system of proportional sampling can be operated; or to have collecting tanks where the whole effluent can be averaged before its discharge into the sewer. The Committee lays stress upon the point that in many existing breweries it would be difficult to establish either of these systems.

(2) *Suspended Solids.*—Considering it undesirable to suggest a limit that could not in fact be achieved in practice, the Committee is of the unanimous opinion that more data are required before a figure can be put forward. Inasmuch as facilities exist under the Act for arrangements between local authorities and industrial concerns for the disposal of effluents, the Committee recommends that use should be made of these facilities, as far as is possible, to arrive at a mutually acceptable figure.

(3) *Yeast.*—The prohibition of yeast in an effluent would make it practically impossible to carry on the business of brewing. The model bye-laws of the Ministry of Health recognise the necessity of admitting some yeast, since they suggest the prohibition of "yeast in excess of . . . ."

The Committee recommends the acceptance of Dr. Ardern's suggestion that yeast should be permitted up to the limit of the suspended solids when such figure shall have been decided; this is in effect a recommendation that the yeast clause should be deleted.

(4) *Sugar.*—The Ministry's model bye-laws recognise that prohibition of sugar would be a hindrance to brewing, since they contain the words "sugar in excess of . . . ." Before a limit could be fixed it would be necessary to define sugar and to agree upon a method of determination. The Turton draft bye-laws and most other proposed bye-laws contain provisions for making a charge, beyond agreed limits, to the industrial undertaking, based on the figure for "oxygen absorbed" from acid permanganate in 4 hours at 80° F. Since excessive sugar content will result in high "oxygen absorbed" figures and will be paid for accordingly, it will be to the interest of the brewer to keep the sugar-content of the effluent as low as possible. The Committee, therefore, recommends non-acceptance of the whole sugar clause.

(5) *Temperature.*—In the Public Health Act, 1936, the discharge of effluents at a temperature higher than 110° F. was prohibited. This restriction is removed in the Public Health (Drainage of Premises) Act, 1937, unless re-imposed by a bye-law. In the opinion of the Committee this temperature limit (110° F.) should be generally accepted for any part of an effluent whether averaged or not. It is mentioned that this restriction is in force under the Public Health (London) Act, 1936.

(6) *Neutrality.*—The *p*H of beer is approximately 4, and still lower for waste beer. Since beer acidity is entirely of organic nature, no serious objection could be taken to its presence in sewers. The Committee therefore recommends a toleration range of *p*H 3·5 to 12 for breweries where the effluent is not averaged.

(7) *Local Agreements.*—In the opinion of the Committee, provided that certain principles are accepted on both sides, much more satisfactory results are likely to be obtained by local agreement than by attempts to conform to limits arbitrarily imposed.

Ministry of Food

THE SAUSAGES (MAXIMUM PRICES) ORDER, 1940*

THE Minister of Food has made this Order in exercise of the powers conferred upon him by Regulation 55 of the Defence (General) Regulations, 1939. It contains 7 Articles, which may be summarised as follows:

1. In this Order:—"Beef Sausages" means sausages which are ordinarily known and sold as beef sausages, and include sausages the meat in which is not beef alone.
   "Pork Sausages" mean sausages which are ordinarily known and sold as pork sausages and include sausages the meat in which is not pork alone.
   "Kosher Beef Sausages" mean beef sausages manufactured as respects the meat therein from beef obtained from cattle slaughtered in accordance with the Jewish practice of slaughter.
   "Meat content" means as respects any sausage the percentage of the weight of meat contained in that sausage relative to the total weight of the contents of the sausage.†
   In relation to sausages:—"Grade A" means sausages or sausage meat having a meat content of not less than 70 per cent. "Grade B" means sausages or sausage meat having a meat content of not less than 45 per cent. and less than 70 per cent.
   "Grade C" means sausages or sausage meat having a meat content of not less than 30 per cent. and less than 45 per cent.
2. This forbids the sale of sausages or sausage meat at a price exceeding the maximum wholesale or retail prices applicable to the respective descriptions given in the Schedule to the Order.
3. Sausages or sausage meat with a meat content of less than 30 per cent. may not be manufactured or sold.
4. Retailers must indicate by means of a ticket or label the grade and meat content of the sausages or sausage meat offered or exposed for sale; it shall be sufficient compliance with the requirements of this Article if the ticket or label is prominently displayed on a slab or tray on which the sausages or sausage meat are exposed for sale.
5. (1) In any prosecution for the sale of sausages or sausage meat with a meat content other than that indicated by the ticket or label mentioned in Article 4, it shall be a defence for the defendant to prove.
      (i) that he purchased the sausages or sausage meat with a written warranty to the effect that the meat content was that indicated on the label or ticket, and
      (ii) that he had no reason to believe at the time of the commission of the alleged offence that such meat was other than that so warranted.
   (2) A warranty shall only be a defence to such proceedings if the defendant has within seven days of the service of the summons sent to the prosecutor a copy of the warranty with a notice stating that he intends to rely upon it, and specifying the name and address of the person from whom he received it, and has also sent a like notice of his intention to that person.
   (3) A servant of the defendant is entitled to rely upon the provisions of this Article in the same way as his employer would have been if he had been the defendant.
   (4) The person by whom the warranty is alleged to have been given shall be entitled to appear at the hearing and to give evidence.
   (5) For the purposes of this Article any statement of grade or meat content in an invoice or similar document relating to any sausages or sausage meat mentioned in that invoice or document shall be deemed to be a written warranty that the sausages or sausage meat to which the statement relates are of the grade of meat content mentioned in the statement.
   (6) Infringements of this Order are offences against Regulation 55 of the Defence (General) Regulations, 1939.
   (7) This Order, which came into force on March 26th, 1940, may be cited as the Sausages (Maximum Prices) Order, 1940.

H. L. FRENCH,
Secretary to the Ministry of Food.

Dated March 18th, 1940.

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* Statutory Rules and Orders, 1940. No. 394. Emergency Powers (Defence). Food. H.M. Stationery Office. Price 1d. net.

† For the determination of Meat Content see ANALYST, 1940, **65**, 257.

Ministry of Health

THE PUBLIC HEALTH (PRESERVATIVES, ETC., IN FOOD) AMENDMENT REGULATIONS, 1940*

THE Minister of Health has made the following Regulations under the Food and Drugs Act, 1938:

1.—These Regulations and the Public Health (Preservatives, &c., in Food) Regulations, 1925 to 1927, shall be construed together and may be cited together as the Public Health (Preservatives, &c., in Food) Regulations, 1925 to 1940. They shall come into force on the date hereof.

2.—The Public Health (Preservatives, &c., in Food) Regulations, 1925 (*a*), as amended (*b*), shall be further amended as follows:

(1) The following additional proviso shall be inserted at the end of Article 4 (1) and at the end of Article 2 (1):

"(iv) The provisions of this Act shall not apply
(*a*) So as to prohibit the presence in bacon, ham and cooked meat of any added sodium or potassium nitrite.
(*b*) So as to prohibit the presence in any article of food of sodium or potassium nitrite introduced in the preparation of such article by the use of any bacon, ham or cooked pickled meat containing sodium or potassium nitrite."

(2) The following paragraph shall be inserted after Article 4 (3):

"(4) No person shall manufacture for sale or sell any cooked pickled meat intended for human consumption, other than bacon or ham, which contains sodium or potassium nitrite in proportions exceeding two hundred parts per million calculated as sodium nitrite."

(3) The following paragraph shall be inserted after Article 11 (2):

"No person shall import into England or Wales any cooked pickled meat intended for sale for human consumption, other than bacon or ham, which contains sodium or potassium nitrite in proportions exceeding two hundred parts per million calculated as sodium nitrite."

(L.S.) J. N. BECKETT,
Assistant Secretary, Ministry of Health.

British Standards Institution

THE following British Standard has been issued†:

NO. 895—1940. METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF BUTTER.

In 1930 the Empire Marketing Board appointed a Committee to consider the standardisation of Methods for the Chemical and Bacteriological Analyses of Milk, Cheese and Butter Samples. This work was taken over by the British Standards Institution in 1933, and the continuance of the work of the Committee was confirmed on November 1st, 1934, at a meeting of the Dairy Standards Technical Committee, with the addition of two new members representing the Society of Public Analysts and Other Analytical Chemists. Methods for the bacteriological examination of butter were reviewed, and it was decided that to be of practical value the methods should be as few and as simple as possible, and should be reproducible, should reflect the commercial properties of butter, reveal faults in manufacture, and afford some measure of the keeping quality. Pathogenic organisms were regarded as outside their purview. A survey of the literature on the

* Statutory Rules and Orders, No. 633. Regulations dated April 30th, 1940. These Regulations supersede the provisional Regulations dated October 20th, 1939. H.M. Stationery Office, 1940. Price 1d. net.
(*a*) S.R. & O. 1925 (No. 775), p. 1375.
(*b*) S.R. & O. 1926 (No. 1557), p. 1177; 1927 (No. 577), p. 455.

† Obtainable from the Publications Department, British Standards Institution, 28, Victoria Street, London, S.W.1. Price 2s. net. Post free 2s. 2d.

bacteriological examination of butter showed that apparently no attempt had been made to establish the reproducibility of results when different workers were applying prescribed methods to the same sample of butter. The Committee decided to repair this deficiency.

The methods recommended are given under the following headings:—(A) GENERAL.—*Method of Sampling—Treatment of Sample—Apparatus—Diluent (Ringer's Solution)—Plating and Counting.* (B) MEDIA FOR THE DIFFERENTIAL ENUMERATION OF VARIOUS MICRO-ORGANISMS IN BUTTER.—*Yeasts and Moulds—Lipolytic (Tributyrin-splitting) Organisms—Caseolytic Organisms—Saccharolytic Organisms—Coliform Organisms.*

These methods are intended as the basis for observations comparing the counts of organisms of the various types with the commercial properties of the butter and revealing faults in manufacture, transport or storage. Those who use the methods are invited to communicate their results to the British Standards Institution.

The Committee recommends storage at 60° F. (as used by the National Mark Butter Committee) for the purpose of observation of keeping quality after cold storage.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Aromatic Principle in Bread. II. Isolation of the Aromatic Principle in Rye Bread. E. Komm and G. Lehmann. (*Z. Unters. Lebensm.*, 1940, **79**, 242–246.)—The bread used in the investigation was a highly flavoured regimental bread two or three days old. Steam-distillation was found unsatisfactory, owing to the high resistance of the bread-pulp, but methyl alcohol, ether, chloroform and acetone extracted the aromatic principle, and methyl alcohol free from acetone was chosen as the most convenient solvent. The finely-crumbled bread (40 kg. in 2000-g. portions) was extracted for 18 hours with 1500 ml. of boiling methyl alcohol. The brownish turbid extract (fraction F_1) was filtered, and a portion when allowed to evaporate at room temperature left a brownish viscous residue with a strong odour of bread. When the extract was distilled *in vacuo* it yielded a distillate (F_2) with an odour resembling that of whisky. This fraction was separated by distillation at ordinary pressure into 6 fractions (F_3 to F_8), at 12 mm. pressure into 4 fractions (F_9 to F_{12}), and at 4 mm. pressure into 10 fractions (F_{13} to F_{22}). None of these fractions indicated any concentration of the aromatic substances. By salting out and extracting F_2 with ether a fraction F_{23} was obtained, and this left on evaporation an acid residue (F_{24}) smelling strongly of bread. Attempts to isolate the aromatic body from this by extraction with solvents failed. F_{24} was neutralised, (F_{25}), saponified for 30 hours with sodium hydroxide, and finally neutralised with hydrochloric acid (F_{26}). This was fractionally distilled, and the fraction with the strongest odour (F_{28}) was further separated into 3 fractions (F_{29} to F_{31}) and F_{30} again into 3 fractions (F_{32} to F_{34}). All these fractions had a strong odour of bread, but the yield was small. F_{26} was evaporated, and the small residue was soaked up with filter-paper, which was then heated in a distillation flask. Three fractions were obtained, of which F_{37} had the strongest odour. The various fractions were examined for the presence of characteristic groups. Carboxylic acids were detected by the thionyl chloride test (Feigl, "*Qualitative Analyse mit Hilfe von Tüpfel Reaktionen*," Leipzig, 1939) in fractions 1 to 24 and 27. Oxy-acids were found in the same fractions by the ferric chloride test, and F_{25} gave a positive result in the Denigès test for lactic acid.

Acetic acid (Feigl's lanthanum nitrate test, *op. cit.*) was present in F_{24}. Phosphoric acid was found in fractions 1 to 3, 9, 13, 23, and 24. The first 30 fractions reduced Fehling's solution. Carbonyl groups were found in F_{28} by the test described in the next abstract. All the fractions answered to Feigl's test (*op. cit.*) for aldehydes with sodium bisulphite, and most of the fractions responded to Fischer and Penzoldt's test for aliphatic aldehydes with diazobenzenesulphonic acid (*Ber.*, 1884, **17**, 572). Angeli's test for aromatic and aliphatic aldehydes (*Gazz. Chim. Ital.*, 1896, **26**, 17) gave positive results with all fractions except F_{17}. Almost all fractions answered to Feigl's test (*op. cit.*) for $\alpha\beta$-unsaturated and aromatic acids with sodium pentacyanoammine ferroate. None of the fractions responded to Legal's test for methyl ketones. Phenols could not be detected, but most of the fractions contained small amounts of higher alcohols. Fusel oil was found in fractions 5 to 8. Since the foregoing tests indicated the presence of unsaturated and aromatic aldehydes in many fractions, individual representatives of these classes were sought for. Zinke and Dietmann's test for furfuraldehyde with a mixture of alcoholic aniline and hydrochloric acid (Gattermann, "*Die Praxis Organischen Chemikers*," Berlin and Leipzig, 1933, p. 374) indicated a high concentration of that substance in F_{37}, the fraction with the strongest odour of the original bread. Most of the other fractions answered to this test. Fiehe's test for hydroxyfurfuraldehyde was given by fractions 8, 12, 27, 28 and 31. The investigation has been interrupted, but from the results already obtained it would appear that the aromatic principle most strongly concentrated in fraction 37 is a substituted furfuraldehyde derivative. A. O. J.

Aromatic Principle in Bread. III. Colorimetric Estimation of Diacetyl, Acetoin and Butylene Glycol. E. Komm and J. Flügel. (*Z. Unters. Lebensm.*, 1940, **79**, 246–250.)—Acetoin (acetylmethylcarbinol) forms a red colour on atmospheric oxidation in presence of 40 per cent. sodium hydroxide solution. Attempts to accelerate the oxidation by using more powerful oxidising agents failed to give a reaction suitable for quantitative purposes. Acetoin and butylene glycol are converted into diacetyl by suitable oxidising agents. Previous work on the estimation of creatinine by means of *m*-dinitrobenzoic acid (Komm and Pinder, *Z. Unters. Lebensm.*, 1939, **78**, 113; Abst., ANALYST, 1940, **65**, 229) suggested that a similar method might be applied to the colorimetric estimation of diacetyl. Experiments with a number of nitro-compounds showed that an alcoholic solution of *m*-dinitrobenzoic acid (1:3:5) was the most convenient reagent, yielding a violet to wine-red colour with cold solutions of diacetyl in presence of alkali. The course of the reaction is influenced by the temperature, the alkali concentration and the duration of reaction. A study of the effects of varying each of these factors in turn led to the following procedure:—The aqueous diacetyl solution (2 ml.) is mixed with 1 ml. of 5 per cent. sodium hydroxide solution, and 2 to 4 ml. of alcoholic dinitrobenzoic acid solution are added so as to form a separate layer. Standard solutions of diacetyl are treated in the same manner. All the reaction mixtures are shaken simultaneously, and the colours are compared after 20 minutes. The method gives satisfactory results with solutions of diacetyl of concentration greater than 1 part in 100,000. It was

used successfully for the estimation of diacetyl, acetoin and butylene glycol in leavened bread. Acetoin and diacetyl were estimated together after oxidation of the acetoin by means of ferric oxide in presence of sulphuric acid. Butylene glycol was converted into acetoin by oxidation with bromine, and then into diacetyl by oxidation with ferric chloride. For diacetyl and acetoin the procedure was as follows:—The finely crumbled bread (150 g.) was distilled in steam, and the distillate was oxidised as described, and again distilled, 60 ml. of distillate being collected. Two ml. of the distillate were used for the colorimetric estimation. For the estimation of butylene glycol, 150 g. of crumbled bread were covered with 300 ml. of water and 10 ml. of conc. sulphuric acid and distilled in steam until 5 litres had been collected. A 500-ml. portion of the distillate was warmed with 1 ml. of bromine on the water-bath for 30 minutes at 70° to 80° C. beneath a reflux condenser. The mixture was rapidly cooled, the bromine was removed by adding finely powdered ferrous sulphate and, after addition of 10 ml. of conc. sulphuric acid and 30 ml. of 30 per cent. ferric chloride solution, the liquid was distilled, 60 to 100 ml. of distillate being collected. The distillate (2 ml.) was used for the colorimetric estimation. Comparative determinations were made by the gravimetric nickel dimethylglyoxime method of Visser't Hooft and de Leeuw (*Biochem. Z.*, **1925**, **161**, 361). The colorimetric method gave somewhat higher results. A. O. J.

Characterisation of Starch from Various Sources by means of Electrophoretic Fractionation. O. Dahl. (*Z. physiol. Chem.*, **1940**, **263**, 81–99.)—A 2 per cent. paste of the starch was treated for 2 to 3 days in an electrophoresis apparatus with an e.m.f. of 40 volts per cm. The sol produced was concentrated under reduced pressure to give a 1 per cent. solution (Amylose I). The gel (Amylopectin I) was diluted with water to re-form a paste, which was re-treated, thereby forming a second sol (Amylose II) and gel (Amylopectin II). A similar treatment of the second gel gave Amylase III and Amylopectin III. The organic phosphorus content of the six fractions prepared from each kind of starch was determined, and also the amount of phosphorylation produced by muscle phosphorylase in a given time was ascertained by measuring the usage of inorganic phosphate. It was found that the organic phosphorus contents of the starches of wheat, rice, maize and potato and of the amylopectin fractions prepared from them were 0·01 to 0·08 per cent., and that phosphorylation proceeded to approximately the same degree with all the starches and their amylopectin fractions. The yields of amylose were 16 to 18 per cent. for wheat-, maize- and potato-starch, and 9 per cent. for rice-starch. The amyloses did not contain any appreciable amount of organic phosphorus, and were phosphorylated to the extent of one-third to one-half the native starches and amylopectins, with the exception of potato-amylose, which was phosphorylated to the extent of only one-eighth. There appeared to be no relationship between the phosphorus-content and the degree of phosphorylation. The anomalous potato-amylose to some extent inhibited the phosphorylation of other substrates. The amylose and amylopectin fractions of wheat- and potato-starch were fractionally digested with α- and with β-amylase, and the extent to which each of the degradation products was phosphorylated was

ascertained. It was found that all the fractions obtained by α-amylase degradation quickly lost their ability to be phosphorylated, whilst the fractions treated with β-amylase did not. F. A. R.

Reducing Power of Unripe Honey and Honey from Sugar-fed Bees. R. F. Kardos. (*Z. Unters. Lebensm.*, 1940, **79**, 258–262.)—The reducing power of honey towards chloramine, under the conditions laid down by Tillmans and Hollatz, was studied as a possible means of discriminating between different classes of honey. This reduction is slight and is due, not to the sugars, but to minute quantities of unknown substances. Ten ml. of 10 per cent. honey solution (10 g. in 100 ml. of solution) were oxidised with 20 ml. of $N/100$ chloramine solution after acidification with 3 ml. of 2 N acetic acid. After the reaction mixture had stood for 10 minutes, dilute sulphuric acid and potassium iodide were added, and the iodine liberated by the excess of chloramine was titrated with $N/100$ sodium thiosulphate solution. The chloramine consumption for sugar-fed honey was found to be 1·1 to 2·3 ml. of $N/100$ per g., which is lower than the range found for normal honey. Unripe honey resembled sugar-fed honey in many of its properties and had a similarly low reducing power (0·9 to 1·9 ml. of $N/100$ per g.). Two samples, although containing no added sugar-fed honey, had exceptionally high sucrose-contents (14·8 and 18·8 per cent.). From this it is concluded that not only sugar-fed honey, but also honey collected by bees brought up on sugar, may contain much sucrose. The reducing power of ripe normal honey ranged from 1·0 to 11·9 ml. of $N/100$ per g. Four samples of ripe, yellow spring honey showed very low reducing powers, and a series of spring honeys gave a range of 1·1 to 2·0. The reducing power should be used with caution as a proof of the presence or absence of sugar-fed honey in a given sample. The reducing power of summer and autumn honey is 3 to 5 ml. (or more) of $N/100$ per g. With sugar-feeding the reducing power is not more than 2 to 3 ml. of $N/100$ per g. In such instances sugar-feeding may be assumed from the lower figure, especially if the sucrose-content, diastatic power and protein-content are within normal ranges. In fresh spring honey a low reducing power does not necessarily indicate sugar-feeding. The first spring honey is separated very early in the year and is often unripe. In winter, when the bee does not collect honey, it apparently loses the power of depositing in the honey the small amounts of biologically important substances that are responsible for the reducing power. For this reason the earliest spring honey (*e.g.* acacia honey) resembles sugar-fed or unripe honey, whereas the "second" acacia honey collected and separated two or three weeks later approaches normal honey in composition. Spring honey that has been gathered by bees artificially fed with sugar during the winter resembles sugar-fed honey. It may be that the bees in inverting are "tired," and also that their brood does not receive correct nourishment. A further reason for the weakness of spring honey may be that the introduction of artificial comb into the hive stimulates comb-building and causes the production of larger amounts of correspondingly weaker honey. It is well-known that the sucrose-content of sugar-fed honey is in general higher than that of normal honey of the same kind, and that on standing the sucrose-content falls more slowly than that of normal honey. A. O. J.

Estimation of Silver in Catadynised Vinegar and Fruit Cordials. O. Noetzel. (*Z. Unters. Lebensm.*, 1939, **78**, 315–321.)—As the catadyn process of sterilising food is now widely used, especially for vinegar and fruit cordials, it is necessary in food control to ensure that the amount of silver present is not too high; it is considered that a quantity of 500γ per litre is sufficient for sterilisation. For estimating the silver the method of Feigl (*Z. anal. Chem.*, 1928, **74**, 380; Abst., ANALYST, 1928, **53**, 615), which depends on the formation of a red silver compound with *p*-dimethyl-aminobenzylidene-rhodanine, is recommended. *Silver in vinegar.*—The organic matter is destroyed either by (1) ignition or (2) a wet process with sulphuric and nitric acids. (1) Evaporate 50 to 100 ml. of the sample in a porcelain crucible, ignite the residue and fuse it with 0·3 g. of a mixture of sodium carbonate and potassium nitrate (1 + 2·5). Dissolve the fused mass in 2 ml. of water and 3 ml. of nitric acid (sp.gr. 1·4) on the water-bath, and evaporate the solution to dryness. Dissolve the residue in 3 drops of nitric acid and 12 ml. of water, add ammonia in excess, boil and filter. Rinse the flask with dilute ammonia, neutralise the filtrate with dilute nitric acid, add 10 per cent. ammonia until the liquid is slightly alkaline, and make up to 30 or 50 ml. Add to an aliquot part 5 ml. of ether and a few drops of a 0·03 per cent. solution of *p*-dimethyl-aminobenzylidene-rhodanine in acetone. In presence of silver a red film of $AgC_{12}H_{11}N_2OS_2$ will be formed at the zone of contact of the liquids. The test is capable of detecting 2γ of silver. A colorimetric estimation may be made by comparison with standards obtained with silver solutions prepared by diluting 4·65 ml. of $N/10$ silver nitrate solution plus 5 drops of nitric acid to 500 ml. (1 ml. = 100γ of silver) or by diluting 1 part of this solution to 10 (1 ml. = 10γ). (ii) For the wet process of destroying organic matter, evaporate 200 to 300 ml. of vinegar to 30 ml., add 30 to 40 ml. of nitric acid (sp.gr. 1·4), and boil until red fumes no longer appear. Add 6 to 7 ml. of conc. sulphuric acid, and boil with dropwise addition of nitric acid until the liquid is colourless. Cool, dilute with a 4-fold volume of water, add ammonia in excess (about 45 ml. of 10 per cent. ammonia solution in all), boil, filter, rinse the flask with dilute ammonia solution, neutralise (to litmus paper), add 1 drop of ammonia solution, and make up 100 ml. Estimate the silver colorimetrically as in (i).

Fruit cordials.—The wet process should be used; 60 to 70 ml. of nitric acid and 7 to 8 ml. of sulphuric acid are required. A shortened process depending on adsorption on activated charcoal may also be used. The fruit cordial (100 to 200 ml.) is diluted with an equal volume of water in a 500-ml. flask, shaken with 0·8 g. of activated charcoal and boiled for 45 minutes, so that its volume is reduced to about a half. After standing for 24 hours the mixture is filtered with the aid of suction in a Buchner funnel (6 cm. in diameter) containing a double layer of filter-paper. The filter and its contents are dried and ignited and the silver in the residue is estimated as described above. D. A.

Mixed Unsaturated Glycerides of Liquid Seed Fats. Some "Non-Drying" Oils. B. G. Gunde and T. P. Hilditch. (*J. Soc. Chem. Ind.*, 1940, **59**, 47–53.)—The acetone solubility method of investigating the glyceride structure breaks down for liquid fats. In fatty non-drying oils, however, with oleic acid

as a preponderant component acid, elaidinisation (geometrical isomerisation) causes transformation of considerable proportions of mixed oleo-glycerides into corresponding mixed elaido-glycerides, which are solid at ordinary temperatures and resolvable to a considerable extent by crystallisation from acetone at 0° C. By using Bertrand's method with selenium at 220° C. as isomerising catalyst (*Chem. Weekblad*, 1936, **33**, 3; *cf.* ANALYST, 1936, **61**, 866) many objectionable by-products are eliminated, but the temperature causes destruction of linolic groups to an undesirable extent, so that figures deduced for the proportions of the component glycerides of the oils examined are of a definitely lower order of accuracy than those previously obtained for solid fats which can be directly fractionally crystallised. Application of the method to two olive oils (from Palestine and Italy), ground-nut and almond oils has indicated that when oleic acid forms less than about 60 per cent. of the total acids, as in ground-nut oil, while saturated and linolic acids are in the neighbourhood of 20 per cent. each, a considerable proportion of the oil may consist of monosaturated-mono-oleo-monolinoleo glycerides with small amounts of monosaturated-dioleins, linoleo-dioleins and triolein. When the oleic acid is much in excess of 60 per cent. of the total acids, as in olive and almond oils, and saturated and linolic acids each amount to less than 15 per cent., mono-oleo-glycerides are present, if at all, in very small proportions, and practically all the linolic acid is present as linoleo-dioleins. In such oils a fairly close approximation to the proportion of component glycerides can be obtained directly from the molar percentage proportions of the component acids by combining each minor component acid in the form of *e.g.* monopalmito-diolein or monolinoleo-diolein, the surplus of oleic acid being present as triolein, and allowance being made for any fully saturated glycerides present. In olive oils the amount of triolein probably rarely exceeds 35 per cent., and in some oils may be almost completely absent. The proportions of component glycerides, approximate, and regarded as probably present in almond oil are myristo-dioleins, 3, palmito-dioleins, 14, linoleo-dioleins, 52, and triolein 31 per cent. (mol.); in ground-nut oil, the combined proportions of the individual saturated acyl dioleins and acyl oleo-linoleins are given as palmito- 30, stearo- 11, arachido- 6, beheno- 7, and lignocero- 2 per cent. (mol.). The probable component glycerides of (*a*) Palestine olive oil and (*b*) Tuscany oil are given as tripalmitin (*a*) 2, (*b*) 2; myristo-diolein (*a*) 2, (*b*) 5; palmito-dioleins, (*a*) 26, (*b*) 38; stearo-dioleins, (*a*) 10, (*b*) 6; hexadeceno-diolein, (*a*) 3, (*b*) 5; linoleo-diolein (*a*) 26, (*b*) 38; triolein (*a*) 31; oleo-linoleo-palmitins, (*b*) 6 per cent. (mol.). The data for (*b*) are not wholly conclusive, and somewhat more mono-oleo-glycerides, together with a small proportion of triolein, might equally well be present. In both olive oils the proportion of triolein itself is far smaller than was formerly supposed. D. G. H.

Determination of the Ascorbic Acid Content of Tablets. E. C. M. J. Hollman. (*Pharm. Weekblad*, 1930, **77**, 393–400.)—The procedure recommended in the Supplement to the Dutch Pharmacopoeia for the determination of ascorbic acid is considered to be unsuitable for use with tablets, which usually contain talc, lactose and starch, and the following method is preferred:—The tablet is rubbed in 2 per cent. acetic acid solution until it is disintegrated, and the mixture

is diluted in a graduated flask to 500 ml. (presumably with the acetic acid.—J. G.). Five ml. of the solution are immediately pipetted into a porcelain dish, 15 ml. of a 3 per cent. trichloroacetic acid solution are added, and the mixture is back-titrated with a standard solution of 2.6-dichlorophenol-indophenol, with agitation, until a rose colour is produced and is permanent for 5 seconds. The indicator solution is prepared by dissolving 20 mg. of the 2.6-dichlorophenol-indophenol in 100 ml. of water, filtering the solution 24 hours later, and adding a little sodium bicarbonate to the filtrate. It should be renewed at least once a week, as if it is kept longer a turbidity develops and a dirty brownish-red colour is produced at the end-point. The importance of minimising the period which elapses between the preparation and titration of the solution of the sample was demonstrated in experiments with a mixture containing 100 mg. of pure ascorbic acid and 150 mg. each of lactose and of starch. Intervals of up to 4 hours were allowed to elapse before titration, and a progressive fall in the titration with lapse of time was found. Similar results were obtained whether water or 2 per cent. acetic acid was used, but when 8 commercial tablet preparations were compared from this point of view, the latter solvent always gave the higher results, and it is therefore recommended for use in the final method. The results indicate that formation into tablets does not cause any reduction in ascorbic acid content (Schou and Bennekou, *id.*, 1938, **75**, 154; Stevens, *id.*, 1938, **75**, 790). J. G.

Biochemical

Estimation of Small Amounts of Sulphur as Sulphates in Biological Fluids. A. D. Marenzi and R. F. Banfi. (*Biochem. J.*, 1939, **33**, 1879–1889.)—An improved method of estimating sulphates in blood and urine has been devised, in which benzidine is used as precipitant and the benzidine is estimated with phosphotungstomolybdic acid. *Sulphur in blood.*—To 1 volume of whole blood, serum or plasma are added 2 volumes of 10 per cent. trichloroacetic acid. The mixture is filtered after standing for 10 to 20 minutes, and 6 ml. of the filtrate are transferred to a 15-ml. conical centrifuge tube. To remove organic and inorganic phosphates, the solution is mixed with 0·5 ml. of 2·5 per cent. aluminium chloride (hexahydrate) solution and 0·5 ml. of ammonia solution (prepared by diluting 20 ml. of ammonia of sp.gr. 0·91 to 100 ml. and adding 5 ml. of 0·02 per cent. methyl red solution). The solution, which should be distinctly alkaline, is allowed to stand until the aluminium hydroxide is flocculated; it is then centrifuged for 5 minutes. Into a second centrifuge tube with a narrow end are introduced 3·5 ml. of the centrifugate, and the solution is neutralised by adding about 1 ml. of freshly prepared 5 per cent. trichloroacetic acid solution in anhydrous acetone. A further 2 ml. of the trichloroacetic acid solution is then added, followed by 6 ml. of a freshly prepared 1·5 per cent. solution of benzidine in anhydrous acetone. After thorough mixing the solution is allowed to stand at 0° C. for 2 hours, and then centrifuged for 15 minutes. The supernatant liquid is decanted, and the centrifuge tube is allowed to drain. The precipitate is washed with 10 ml. of acetone containing 5 per cent. of trichloroacetic acid, the liquid is decanted and the tube drained as before, and the precipitate is dissolved in 2 ml. of warm 0·2 *N*

hydrochloric acid. The solution is transferred to a 25-ml. graduated tube with the aid of 3 or 4 small portions of water, so that the final volume is 6 to 8 ml. The benzidine solution is treated successively with the following reagents, the tube being thoroughly shaken after each addition: (*a*) 0·5 ml. of 1 per cent. gum arabic solution (treated before use with a few drops of bromine, the excess of which is expelled by boiling), (*b*) 1 ml. of phosphotungstomolybdic acid* (Folin and Ciocalteu, *J. Biol. Chem.*, 1927, **73**, 627), (*c*) (after 3 minutes) 2·5 ml. of 16 per cent. sodium carbonate solution, and finally after an interval of at least 10 minutes, to allow the alkali to destroy the excess of reagent, (*d*) 2 ml. of recently prepared 2 per cent. sodium sulphite solution. This converts the indefinite green colour of the reaction mixture into a stable blue-grey colour; after a few minutes the solution is made up to 20 ml., and the colour is compared with that given by known amounts of benzidine hydrochloride treated in a similar manner. For greater accuracy, the colour may be measured in a Pulfrich photometer with filter S72. The result indicates the amount of *inorganic sulphate* present. To estimate the *total sulphate*, 6 ml. of blood filtrate and 0·5 ml. of *N* hydrochloric acid are evaporated to dryness in a small beaker, the residue is dissolved in 6 ml. of water, and 0·5 ml. of 2·5 per cent. aluminium chloride solution and 0·5 ml. of the ammonia and methyl red solution are added. After centrifuging, 3·5 ml. of the supernatant liquid are treated as described above. The *total sulphur* is estimated by digesting 3 ml. of blood filtrate with 3 ml. of pure conc. nitric acid in a 100-ml. Kjeldahl flask until the liquid turns brown. A few drops of perhydrol are added, and the heating is continued until a white ash remains. This is dissolved in 6 ml. of water and treated with aluminium chloride solution and ammonia, as described above.

Sulphur in urine.—One ml. of urine of normal concentration is transferred to a 50-ml. volumetric flask, and 4 ml. of 0·4 per cent. uranyl acetate solution are added to remove phosphates. After shaking, the flask is allowed to stand for at least 5 minutes, and the solution is then diluted to a definite volume and filtered. To estimate *inorganic sulphate*, 1 ml. (equivalent to 0·02 ml. of urine) is treated with 2 ml. of 10 per cent. trichloroacetic acid solution, and the estimation is continued as for blood. *Total sulphate* is determined on 0·5 ml. of the filtrate; this is treated with 2 to 3 ml. of water and 0·5 ml. of *N* hydrochloric acid and further treated as described for blood. The *total sulphur* is estimated by incinerating 1 ml. of the filtrate with nitric acid and perhydrol as described above.

The colour developed is strictly proportional to the amount of benzidine present. Inorganic sulphate, added to blood and urine, was recovered with an error not exceeding 4 per cent. F. A. R.

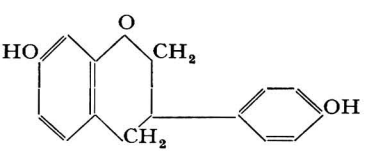
Urothione, a Yellow Sulphur-containing Pigment from Human Urine. W. Koschara. (*Z. physiol. Chem.*, 1940, **263**, 78–79.)—A lemon-yellow pigment, to which the name urothione has been given, was isolated from human urine in a

* The phosphotungstomolybdic acid reagent is made by mixing 100 g. of sodium tungstate (dihydrate) and 25 g. of sodium molybdate (dihydrate) with 700 ml. of water, adding 50 ml. of 85 per cent. phosphoric acid and 100 ml. of conc. hydrochloric acid to the mixture, and boiling under reflux for 10 hours. At the end of this period 150 g. of lithium sulphate, 50 ml. of water and a few drops of liquid bromine are added, and the solution is boiled for 15 minutes to expel the excess of bromine. It is then cooled, diluted to 1 litre and filtered. The final solution should be free from any green tint and should be kept protected from dust, as organic materials gradually produce slight reduction.

yield of 40 to 80 mg. per 1000 litres. It contains 20 per cent. of sulphur, and has no definite m.p. It is very soluble in water, alcohol and acetone, dissolves readily in acids and alkalis, but is insoluble in ether and chloroform. When its aqueous solution is layered with conc. sulphuric acid, a cherry-red ring is formed at the junction of the two phases. A solution of the new pigment in sulphuric acid exhibits no fluorescence in ultra-violet light, but after oxidation with potassium permanganate, and removal of the excess with hydrogen peroxide, the solution fluoresces olive-green. This test gives a positive result with a dilution of 1 part in 10 millions, and can actually be demonstrated with urine. Urothione has the formula $C_{11}H_{13}O_3N_5S_2$ and yields one molecule of methyl mercaptan on catalytic hydrogenation; it contains one free amino-group (van Slyke) and gives a tetra-acetyl derivative, m.p. 220° C. The pigment also occurs in the liver and certain other organs of man and cattle, but nothing is known of its physiological significance.

F. A. R.

Colour Reaction for the Detection of the Equol of Mare's Urine. W. Dirscherl. (*Z. physiol. Chem.*, **1940**, **264**, 57–63.)—When certain fractions from mare's urine were layered with conc. nitric acid, a red ring appeared at the junction of the two liquids, and on heating similar extracts with an equal volume of 25 per cent. nitric acid, a red precipitate was produced, which dissolved in ether or amyl alcohol to form red solutions. The red precipitate was also soluble in sodium hydroxide solution and in ammonia, and was re-precipitated on addition of acid. The coloured product was not produced by other acids. The substance in the extracts of mare's urine responsible for the reaction was shown to be equol (7,4′-dihydroxyisoflavan),



The reaction, which will detect 0·1 per cent., can be made even more sensitive (0·01 per cent.) by evaporating a drop of the solution with a drop of nitric acid on a microscope slide and examining the resulting solid under the microscope. Other phenols, including the oestrogenic hormones, produced either a yellow colour or no colour at all, and even the closely related substances daidzein (7,4′-dihydroxyisoflavone) and its methyl ether, formononetin, gave a negative result. Many other substances, such as chromans, coumarans, flavanones and xanthones, were tested, but only equol gave the characteristic coloured precipitate. Whereas α-tocopherol gives a red colour with nitric acid in alcohol solution (Furter and Meyer, *cf.* ANALYST, 1939, **64**, 217) equol gives a yellow colour, and in small amounts does not interfere with the estimation of tocopherol. Conversely, tocopherol does not give a colour in aqueous solution. The equol derivative responsible for the red colour is believed to be a nitro-quinone. F. A. R.

Studies on the Anti-Grey Hair Vitamin for Silver Foxes. G. Lunde and H. Kringstad. (*Saertrykk av Norsk Pelsdyrblad*, **1939**, **13**, 500–505; *Naturwiss.*, **1939**, **45**, 755). In previous experiments it was demonstrated that

rats require a vitamin B_x belonging to the vitamin B complex, the absence of which causes the black colour of the fur to change to grey. The same colour change has now been shown to take place in fox cubs deprived of vitamin B_x, the change being especially evident on the snout. The cubs fed on the vitamin B_x-deficient diet (basal diet with marmite and synthetic aneurin) were noticeably more docile than cubs fed on a complete diet. The new vitamin is heat-labile, and is not adsorbed by fuller's earth. F. A. R.

Combined Ascorbic Acid of Animal Tissue. P. Holtz. (*Z. physiol. Chem.*, 1940, **263**, 187–205.)—Ascorbic acid occurs in animal tissues and body fluids not only in the free state, but also combined with protein. In the usual method of estimation, that is, after removal of protein, only the free ascorbic acid is estimated. The combined ascorbic acid can be estimated by breaking down the protein-vitamin complex, by hydrolysis with hydrochloric acid or by the action of a proteolytic enzyme such as pepsin or papain, or by autolysis. Of these methods, acid hydrolysis is the most satisfactory. The protein precipitate obtained from the tissue extract or body-fluid by treatment with sulphosalicylic acid or trichloroacetic acid is heated for 10 minutes at 100° C. with 0·5 *N* hydrochloric acid in an atmosphere of carbon dioxide. The solution is then made up to a definite volume with sulphosalicylic acid or trichloroacetic acid solution respectively and filtered or centrifuged, and the clear filtrate is used for the titration. Combined ascorbic acid appears to be the reserve form of the vitamin, and is much more stable to oxidation than the free form. F. A. R.

Tillmann Reaction for Vitamin C in Plant Extracts. C. Gatti and A. Knallinsky. (*Z. physiol. Chem.*, 1940, **263**, 37–40.)—Experiments with guinea-pigs showed that maté leaves had no protective action against scurvy, nor had an infusion prepared from them. On titrating extracts with 2:6-dichlorophenol-indophenol, however, a reduction value equivalent to 15 to 18 mg. of ascorbic acid was obtained. Solutions of medicinal tannin also reduced the indicator, and since maté contains from 4 to 20 per cent. of tannin-like substances, only a portion of which is precipitated by lead acetate or formaldehyde, it seemed possible that these substances in maté were responsible for its reducing action. Maté was soaked for 20 minutes with cold water and filtered, and the extract was titrated at intervals with the indicator. A steady rise in the titre was observed over a number of days, whereas the reducing power of a solution of ascorbic acid steadily decreased. A solution of tannin heated to 95° C. and then titrated with the indicator gave a high reduction value, thereby behaving like hot aqueous extracts of maté and China tea, but differently from ascorbic acid solutions. A value equivalent to 40 mg. of ascorbic acid per 100 g. was obtained with China tea, although this is known not to possess antiscorbutic activity. The tannins of China tea were completely removed by two or three precipitations with lead acetate and sodium sulphate, and the final filtrate did not reduce 2:6-dichlorophenol-indophenol. The tannin-like substances of maté, however, were much more difficult to remove, and some remained even after four precipitations. The following simple method of freeing maté extracts from tannin substances was used:—One g. of hide powder was added to each 10 ml. of infusion, and, after standing for three hours, the

mixture was treated with lead acetate and filtered, and the precipitation with lead acetate was repeated. The filtrate gave a negative reaction for tannins and did not reduce the indicator. Thus the Tillmann reaction is not applicable to the estimation of ascorbic acid in plant extracts, such as tea and maté, which contain tannins or allied substances. These must be removed before the titration is carried out. F. A. R.

Colorimetric Determination of Tocopherol (Vitamin E). IV. Quantitative Determination of Tocopherol in Oils after Saponification. A. Emmerie. (*Rec. trav. chim. Pays-Bas*, 1940, **59**, 246–248.)—The stability of tocopherol towards methyl alcoholic potassium hydroxide solution is affected by the amount and concentration of the alkali solution used, and by the temperature and the time of heating. The conditions for saponifying wheat-germ oil and olive oil to produce the minimum destruction of tocopherol were determined. It was found that saponification was complete when 1 g. of wheat-germ oil was heated with 2 ml. of 2 *N* methyl alcoholic potassium hydroxide solution at 72° to 74° C. for 10 minutes, and that the maximum amount of tocopherol was thereafter extracted. The saponifications were carried out in test-tubes (16 × 140 mm.), fitted with small reflux condensers. After saponification 8 ml. of absolute methyl alcohol and 10 ml. of water were added, and the mixture was extracted with three 50-ml. portions of peroxide-free ether. The combined ethereal extracts were washed with dilute alkali and water, dried over pure sodium sulphate and evaporated in an atmosphere of carbon dioxide. The residue was dissolved in a suitable volume of ethyl alcohol or benzene, and the amount of tocopherol was measured by the colour reaction previously described (*cf.* ANALYST, 1939, **64**, 216, 446, 837). It should be noted that this reaction should be carried out in very subdued daylight or in very weak artificial light. Tocopherol added to either wheat-germ oil or olive oil was recovered by this method to the extent of 98 per cent. F. A. R.

Bacteriological

Bacteria from Chlorinated Waters. M. Levine, P. Carpenter and J. M. Coblentz. (*J. Amer. Water Works Ass.*, 1939, **31**, 1511–23; *Bull. Hygiene*, 1940, **15**, 129–130.)—Bacteriological examinations of the chlorinated water from Lake Michigan yielded 282 strains of the coli-aerogenes group—67 in the winter and 215 in the summer months, the residual chlorine ranging from 0·2 to 0·4 p.p.m. Of these strains, 28 were *B. coli*, 85 *B. aerogenes* and 83 intermediates. Many were obtained from presumptives showing poor gas formation. The question of the sanitary significance of these organisms is of great importance. The resistance of suspensions of cultures of these organisms to chlorine was tested. Suspensions were made from agar slope cultures and adjusted to about a million organisms per ml., chlorine was added to make a concentration of 0·4 p.p.m., and the number of surviving organisms and the residual chlorine (usually 0·2 p.p. 10^6) were determined after 20 minutes. None of the winter strains, and only a few of the summer strains, showed any resistance to chlorine. The authors conclude that there is no inherent resistant character of individual organisms, but only resistance

associated with clump formation or protection by some constituent or character of the water. As already stated, 196 (28 + 85 + 83) of the 282 strains were of the coli-aerogenes group; the remainder were non-lactose fermenters and related to the typhoid-paratyphoid-dysentery group, although not absolutely agreeing with the cultural characteristics of these pathogens. One typhoid-like strain agglutinated with typhoid serum at a titre of 1:1000. The survival of these bacteria may indicate the possible survival of pathogenic members of the same genus. It appears therefore that a residual chlorine-content of 0·2 to 0·4 p.p.m. is not an absolute guarantee of effective sterilisation under all conditions.

D. R. W.

Stability of Bacteria in Relation to *p*H. J. G. Baumgartner and G. G. Knock. (*J. Soc. Chem. Ind.*, 1940, **59**, 53–56.)—The five bacterial species *Esch. coli, Esch. cloacae, Proteus vulgaris, Staph. aureus* and a *Lactobacillus* strain, regarded as protein systems, were used to ascertain the optimum *p*H with regard to (*a*) heat resistance, (*b*) precipitation with ethyl alcohol, and (*c*) flocculation as measured by opacity of the bacterial suspension. Except with the *Lactobacillus* sp., which was grown for 4 days on wort-agar, the suspensions were obtained from 24-hour agar cultures, and the organisms were removed, washed and suspended in water, shaken for 5 minutes, and filtered through cotton-wool pulp. McIlvaine's phosphate-citric acid buffer was used, diluted 1 in $2\frac{1}{2}$, and 100-ml. quantities were prepared for each *p*H value from 3·0 to 8·0. (*a*) The heat treatment was carried out by adding 1 ml. of the suitably diluted aqueous suspensions of the bacteria to 25 ml. of the buffer solutions, and, after mixing, 3 ml. of each suspension were heated in thin-walled tubes in a water-bath. After rapid cooling in water 1 ml. of the suspension and 1 ml. of a 1 per 1000 dilution were plated. (*b*) For the alcohol treatment 5 ml. of absolute alcohol were added to 20 ml. amounts of buffer solution, adjustments of the *p*H were made when necessary, and the *p*H values were determined electrometrically at the end of the experiment. The bacteria were added to the alcohol-buffer mixture and held at 22° C., and at the end of the exposure 1-ml. amounts were placed in 19 ml. of sterile water, and plates were prepared. (*c*) To test the stability in suspension, thick suspensions of the bacteria were made in the buffer solution, 20-ml. amounts being taken in standard tubes ($6 \times \frac{3}{4}$ in.), which were held at 4° C. for 24 hours, and the degree of opacity and sedimentation were estimated visually, *i.e.* the point at which opacity was maximal and sedimentation minimal. In each set of tests excellent agreement between the five bacterial species was obtained with regard to the optimum *p*H, so that at this point for a given bacterial suspension a common factor in stability appears to be operating, and this is believed to be hydration of the bacterial protein. Three tables are given showing details of experiments. It has not yet been ascertained why different bacterial species have a critical *p*H value ranging, in the five species examined, from 5·2 to 7·2.

D. G. H.

Agricultural

Determination of the Size Distribution of Soil Clods and Crumbs. E. W. Russell and R. V. Tamhane. (*J. Agric. Sci.*, 1940, **30**, 210–234.)—A method of simple sieving will give the required information, provided that the

soil is not too wet; if it is, individual clods smaller than 3 mm. will stick together on the 3-mm. sieve. The method and technique should be varied according to the information required. If many of the crumbs are larger than 0·5 mm., a water-sieving method is essential. For general purposes it is desirable, when possible, to use a very slow or a vacuum-wetting technique and a very rapid wetting technique, such as wetting the soil by immersion in water. When most of the water-stable crumbs are smaller than about 0·1 mm., the hydrometer method will be adequate unless separated crumb fractions are required; elutriation is then essential. For general purposes the use of air-dry soil is recommended. Very lumpy soils must be sieved before it is possible to take a 100-g. sample; pre-sieving on a $\frac{5}{8}$-inch sieve facilitates good sampling. The distribution curves for the clods and the residual soil should be determined separately, and that for the whole soil calculated from them. It has been found possible to separate the crumb fractions of Rothamsted soils into crumbs and sand particles by dispersing them in a mixture of bromoform and carbon tetrachloride of the correct density; the crumbs float, while the sand particles sink. There is always some loss of soil in this method, but it is usually less than 5 per cent. A rapid means of overcoming the difficulty of distinguishing between crumbs and sand particles is to determine the proportion of the total base exchange capacity of the soil present in each crumb fraction. For this purpose the rapid method of Schofield (*J. Agric. Sci.*, 1933, **23**, 252) is recommended. The degree of aggregation of a soil is usually defined as that proportion of the soil or of some soil component which is in crumbs larger than a certain size d. There is no optimum choice for the crumb size of d; the value $d = 0{\cdot}5$ mm. has usually been chosen at Rothamsted as affording the most information about the different soils studied.

Distribution of Manganese in the Pea Seed in Relation to Marsh Spot. H. H. Glasscock and R. L. Wain. (*J. Agric. Sci.*, 1940, **30**, 132–140.)—The disease known as marsh spot is confined to the seed of the culinary pea, and its occurrence is noted yearly in certain districts in this country and elsewhere in Europe, especially Holland. The cells of the affected areas die and form a lesion, involving the cotyledons, which show spots having a water-soaked appearance. Previous investigators have failed to associate the cause of the disease with fungi, bacteria or a virus. The present work indicates that the cause is a deficiency of available manganese in the soil. For the determination of manganese, the colorimetric method of Willard and Greathouse (*J. Amer. Chem. Soc.*, 1917, **39**, 2366; Abst., ANALYST, 1918, **43**, 44) was used, and for whole peas the procedure of Richards (ANALYST, 1930, **55**, 554) was adopted. In the diseased peas the highest amount of manganese (5 p.p.m.) was found in the peripheral tissues of the cotyledons followed by the germ (3 p.p.m.) and seed-coat (2 p.p.m.). The figures for a healthy sample were 15 p.p.m. in the germ, 11 p.p.m. in the outer tissues of the cotyledon, and 4 p.p.m. in the seed coat. A given weight of small peas contained less manganese than the same weight of large peas selected from the diseased sample, whereas the reverse was found for groups of similar size from the healthy sample. Thus in the diseased large peas the amounts of manganese ranged from 4·0 to 4·3 p.p.m., and in the small peas from 3·3 to 3·5 p.p.m., whilst in the healthy

peas the range was from 8·3 to 9·3 p.p.m. (average 8·7) for large seeds and from 8·4 to 9·9 p.p.m. (average 9·3) for small seeds. It is suggested that migration of cell contents from the necrotic tissue of diseased peas may partly account for the differences in the manganese-contents of healthy and diseased peas.

Determination of Pyrethrin I. The Seil Colour Reaction in the Mercury Reduction Method. C. S. Sherman and R. Herzog. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 136–137.)—Wilcoxon's method (*Contrib. Boyce Thompson Inst.*, 1936, **8**, 175–181) and Holaday's revised method (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 5) are based on the interaction of pyrethrin I acid and Denigès' reagent (acid mercuric sulphate). Both methods give erratic results, owing to the presence of substances other than pyrethrin I acid which interfere with the titration of the resulting mercurous chloride with potassium iodate. To obtain readable end-points, excess of filter-paper in the titration mixture must be avoided. The colours accompanying the reaction (Seil's colour reaction) appear to be due to the formation of a colloidal dispersion of metallic mercury, or of some mercury compound, which on standing undergoes spontaneous successive increases in particle size until ultimately a coarse blue suspension results. Sodium chloride was found to be the best precipitant for complete conversion of the mercurous ion into calomel and for elimination of fine suspended material. Provided that the mixture is centrifuged for a short time after addition of the sodium chloride, it is not necessary to allow more than 15 minutes between adding the Denigès reagent and the sodium chloride. D. G. H.

Organic

***o*-Nitrobenzazide as a Reagent for the Identification of Phenols. P. P. T. Sah and W.-H. Yin.** (*Rec. Trav. Chim. Pays-Bas*, 1940, **59**, 238–245.)—In boiling ligroin solution *o*-nitrobenzazide decomposes to give gaseous nitrogen and *o*-nitrophenyl isocyanate, the latter reacting with phenols to form crystalline *o*-nitrophenyl urethanes, which can be used to identify the phenols. The azide (0·5 g.) is mixed with a molecular equivalent of the phenol in a small Erlenmeyer flask, covered with 5 ml. of anhydrous ligroin, and heated on a sand-bath under reflux for 4 to 5 hours. With ortho-substituted phenols 2 drops of freshly distilled dimethylaniline are added as catalyst. After cooling and standing overnight the urethanes are filtered off under suction and then recrystallised from suitable solvents (usually ligroin, sometimes ethyl acetate, and occasionally a mixture of the two). Tables show the melting-points, crystalline forms and nitrogen-contents of the *o*-nitrophenyl urethanes prepared from 28 phenols, and also a comparison of the melting-points of the *o*-, *m*-, and *p*-nitrophenyl urethanes from the same phenols. In general, the *para*-isomers have the highest melting-points and the lowest solubilities, and the *ortho*-isomers the lowest melting-points and highest solubilities, the values for the *meta*-isomers coming between these. The exceptions noted are (1) 2.4.6-trichlorophenol and 2.4.6-tribromophenol, which yield *m*-nitrophenyl urethanes with slightly higher melting-points than those of the *p*-nitro isomers; (2) hydroquinone monomethyl ester, the *o*-nitrophenyl urethane

from which has a higher m.p. than the *m*-nitro isomer, though lower than that of the *p*-nitro isomer; (3) *o*-iodophenol, the *o*-nitrophenyl urethane from which has a higher m.p. than those of the *m*-nitro and *p*-nitro isomers. E. M. P.

p-Nitrobenzazide and p-Nitrophenyl Isocyanate as Reagents for the Identification of Amines. P. P. T. Sah. (*Rec. Trav. Chim. Pays-Bas*, 1940, **59**, 231–237.)—In boiling anhydrous toluene solution *p*-nitrobenzazide decomposes to form *p*-nitrophenyl isocyanate; this reacts with amines to yield crystalline *p*-nitrophenyl ureas which can be used to identify the amines. The nitrobenzazide (0·19 g.) or an equivalent quantity of *p*-nitrophenyl isocyanate (prepared by refluxing *p*-nitrobenzazide in absolutely dry toluene and in complete absence of moisture, distilling under vacuum, and collecting and recrystallising from carbon tetrachloride the portion boiling at 160–165° C. at a pressure of 18–20 mm.) is dissolved in 5 ml. of anhydrous toluene in a small Erlenmeyer flask. Slightly more than a molecular equivalent of the amine is dissolved in 5 ml. of dry toluene, and the solutions are mixed and heated under reflux on a sand-bath for 4 hours. After standing overnight the *p*-nitrophenyl urea is filtered off under suction and recrystallised from a suitable solvent (usually 95 per cent. ethyl alcohol, sometimes acetone, ethyl acetate, ligroin, or benzene), and the melting-point is determined. Tables show the melting-points, crystalline forms and nitrogen-contents of the *p*-nitrophenyl ureas from 50 amines. In general, primary amines give the best results; the ureas from secondary amines are rather soluble in toluene, so that concentration of the solvent or the use of ligroin is necessary to isolate the pure urea; amides and anilides also react, but the yields are decidedly less and the products are more difficult to purify. For some of the ureas it was necessary to use as the melting-bath liquid the alkyl esters of phthalic acid (Sah and Kao, *J. Chinese Chem. Soc.*, 1937, **5**, 86–88; Brown, *J. Chem. Education*, 1937, **14**, 380) with which it is possible to determine melting-points as high as 320° C.

E. M. P.

Little-known Reaction for Benzoic Acid. N. Schoorl. (*Pharm. Weekblad*, 1940, **77**, 425–427.)—The method is a modification of the reactions of Guerbet (ANALYST, 1920, **45**, 334; 1921, **46**, 11) and of Guignes (*Bull. Soc. Pharmacol.*, 1928, **35**, 292), in which the benzoic acid is converted into *m*-nitrobenzoic acid, which is reduced to *m*-aminobenzoic acid, and this is diazotised and coupled with β-naphthol to produce a coloured compound. The sample is nitrated with 1 drop of fuming nitric acid (sp.gr. 1·50), and the mixture is evaporated to dryness on the water-bath. The yellow residue is washed into a test-tube with a few drops of 4 *N* sodium hydroxide solution, and reduced by addition of a few drops of a 10 per cent. solution of stannous chloride in 4 *N* hydrochloric acid. The solution is warmed and then cooled, a piece of aluminium is added and cooling is continued; the object of this is to precipitate the tin, a large excess of which interferes with the subsequent formation of the colour. A few drops of 1 per cent. sodium nitrite solution are then added, and the solution is shaken and made alkaline with an ammoniacal solution of β-naphthol. In presence of benzoic acid a bright red colour develops; sensitiveness, 0·1 mg. Cinnamic acid produces the same colour, since the nitric acid oxidises it to benzoic acid. Salicylic acid and *p*-hydroxybenzoic acid give

a dark brown and a dark-red brown precipitate, respectively. If, however, it is desired to test for benzoic acid in presence of these, they may be oxidised with an alkaline solution of potassium permanganate, which does not affect the benzoic acid. If the solution is then acidified with sulphuric acid, the benzoic acid may be extracted with ether. This method, however, does not distinguish benzoic acid from cinnamic acid, since the two acids behave similarly (see also Pesez, *Pharm. Weekblad*, 1940, **77**, 403). J. G.

Essential Oil of Massoi Bark. T. H. Meijer. (*Rec. Trav. Chim. Pays-Bas*, 1940, **59**, 191–201.)—The principal oil-containing barks of Dutch New Guinea, Massoi and Lawang, may be distinguished by the fact that Lawang oil contains eugenol and therefore gives a greenish colour with aqueous ferric chloride in alcoholic solution, whereas Massoi oil does not. Abe (*J. Chem. Soc. Japan*, **1937**, **58**, 246–251), who was the first to investigate real Massoi oil, found that it had the following characteristics: n_D^{20} 1·4721, sp.gr. at 20°/20° C. 0·9822, and optical rotation $\alpha_D = -87{\cdot}01°$. The author obtained the following values for authentic samples of the oil:

n_D^{20}	1·4770	1·4726	1·4717	1·4749
Sp.gr. at 27·5°/4° C. ..	0·9821	0·9643	0·9695	0·9782
Optical rotation α_D =	−94°	−64°	−74°	−106°

The yield of essential oil varied between 1·7 and 2·4 per cent. The constants of two samples of genuine Lawang oil were: n_D^{20}, 1·534 and 1·5350; sp.gr. at 15°/15° C., 1·060 and 1·0559; rotation, −0·9° and −0·8°; eugenol, 60 and 94 per cent.

Massoi oil contains a lactone which on hydrogenation takes up one molecule of hydrogen; oxidation of the dihydrolactone with potassium dichromate and sulphuric acid yields valeric, caproic, succinic, glutaric and δ-keto-capric acids. Caproic acid is also obtained by oxidising Massoi oil lactone with permanganate or with dichromate and sulphuric acid; with the latter reagents fumaric acid is also formed. E. M. P.

(*Note.*—Oil from the Massoi bark of commerce has been found to correspond more closely with the Lawang oil as described above.—EDITOR.)

Determination of Hydrocarbons in Unsaponifiable Matter. J. Grossfeld (*Z. Unters. Lebensm.*, 1939, **78**, 273–285.)—On shaking 3 ml. of potassium hydroxide solution (sp.gr. 1·50), 20 ml. of 96 per cent. alcohol, 20 ml. of water and 50 ml. of benzine (b.p. 60–70° C.) with a weighed quantity of paraffin (paraffin-wax or mineral oil), and, after separation of the phases, evaporating 25 ml. of the benzine solution, recovery of the paraffin is quantitative. With cholesterol there is a 98 per cent. recovery. If, however, palmitic acid is added, paraffin and cholesterol behave differently. Under these conditions the proportion of paraffin (calculated on 50 ml. of benzine) becomes 109 per cent., and that of the cholesterol only 26 per cent. The apparent high yield of paraffin is due to the diffusion of benzine into the soap solution; this amounts to 7 ml., so that the concentration of paraffin in the residual 43 ml. is increased. A new analytical constant, the hydrocarbon value (HCV),* has been based on this difference. A weighed quantity of the unsaponifiable matter (*e.g.* 0·5 g.) is mixed with 5 g. of a fatty acid (conveniently palmitic or

* HCV is used here to represent KWZ (Kohlenwasserstoffzahl) in the German original paper.

oleic acid), 20 ml. of 96 per cent. alcohol, 3 ml. of potassium hydroxide solution (sp.gr. 1·50) and a few granules of pumice, and boiled for 5 minutes beneath a reflux condenser. The mixture is then cooled to about 38° C. if palmitic acid was used or to about 15° C. for oleic acid, and gently mixed with 50 ml. of benzine (b.p. 60°–70° C.). After further cooling if palmitic acid was used, 20 ml. of water are added, and the flask is closed with a rubber stopper, shaken and allowed to stand overnight. Twenty-five ml. of the benzine solution are then transferred to an Erlenmeyer flask by means of a pipette, the benzine is distilled off, and the residue is dried at 105° to 110° C. and weighed. A control test is made simultaneously with the reagents alone, and the amount of residue obtained is deducted from the residue containing the unsaponifiable matter. The following constants (P.HCV = palmitate hydrocarbon value; O.HCV = oleate hydrocarbon value) were determined:

	Paraffin	Sterol	Cetyl alcohol
P.HCV ..	109	26	39
O.HCV ..	107	27	43

For calculating the amount of paraffin in presence of sterol only, the formula is:

$$x = 1{\cdot}21\ (\text{P.HCV}-26) \text{ or } 1{\cdot}25\ (\text{O.HCV}-27).$$

If only cetyl alcohol is present with paraffin,

$$x = 1{\cdot}43\ (\text{P.HCV}-39) \text{ or } 1{\cdot}56\ (\text{O.HCV}-43).$$

For mixtures of cetyl alcohol and sterol the amount of the former is calculated by the formula: $C = 7{\cdot}7\ (\text{P.HCV}-26)$ or $6{\cdot}25\ (\text{O.HCV}-27)$. When the unsaponifiable matter contains the three components, one must be determined separately, *e.g.* sterols by precipitation with digitonin. The two others can then be calculated from the hydrocarbon value. For example, in a test experiment in which a mixture of 0·120 g. of paraffin, 0·210 of cetyl alcohol and 0·155 g. of cholesterol was taken, the amount of paraffin found was 0·119 g. D. A.

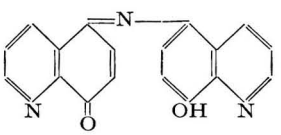
Application of Rational Analysis to some Typical South African Coals. G. K. Morrison. (*J. Chem. Metall. Mining Soc. S. Africa*, 1939, **40**, 201–212.)—The main constituents of coal from the point of view of rational analysis are the hydrocarbons and resins, the organised plant entities (spore exines, cuticles, etc.), the ulmins, and fusain. Francis and Wheeler (*J. Chem. Soc.*, 1928, 2967) have shown, however, that, since no correlation exists between the proportion of these constituents and the maturity and rank of a coal, no system of classification can be derived from them; more information is obtainable from the reactivity of the ulmins towards oxygen. In determining the reactivity the hydrocarbons and resins are first removed by extracting the sieved coal (passing 60 and retained on 120 I.M.M. sieve) with boiling pyridine for 8 hours. If most of the solvent is then removed from the extract, and the residue is acidified, thoroughly dried and extracted with dry ether, the residue, after evaporation of the ether, gives the hydrocarbons and resins. Although this extraction process is necessary and satisfactory as a preliminary to the determination of ulmin activity, it is unsatisfactory as a method for the determination of hydrocarbons and resins, owing to the dependence of the results on the purity of the pyridine and to

the slow removal of the hydrocarbons and resins from the pyridine extract. The (resistant residue + fusain) is obtained by boiling 0·5 g. of the sieved extracted coal (passing 120 and retained on 150 I.M.M. sieve) for 7 hours under a reflux condenser with 50 ml. of oxidising solutions (mixtures of potassium chlorate and *N* nitric acid) of different strengths. With the aid of a microscopical examination of the residue, the oxidising agent can be chosen so that the ulmins are dissolved out, whilst the resistant plant entities are unattacked. The weight of residue after oxidation (*P* g.) gives therefore, the amount of resistant residue plus the insoluble ash in the coal. With the four South African coals examined, however, there were indications that portions of the resistant residue are attacked before all the ulmins have been rendered soluble, or that a modified form of ulmin is present, the oxidisability of which overlaps that of the true ulmin and that of the resistant residue. The method therefore is only approximate as a means of classification, and it is usually preferable to determine the resistant residue in the clarain (*cf.* Francis and Wheeler, ANALYST, 1931, **56**, 333, 420); here again, however, this presented difficulties with the South African coals because of their small clarain contents. An alternative measure of the resistance of ulmins to oxidation, and consequently of the maturity of a coal, is Francis and Wheeler's "reactivity index." This is the percentage of the ulmins rendered soluble by oxidation of 0·5 g. of the coal (after extraction with pyridine, as described) with a solution of 0·9 g. of potassium chlorate in 50 ml. of *N* hydrochloric acid. The reaction is carried out in a pressure-bottle, which is immersed in boiling water for 7 hours and then allowed to cool over-night, the oxidised ulmins being dissolved out with *N* potassium hydroxide solution and the insoluble residue dried and weighed (*R* g.). If the weights of ash from 0·5 g. of coal after extraction with pyridine and from the above *R* g. of residue are *A* and *A'*, respectively, then the reactivity index is given by $100\ [(0{\cdot}5 - A)-(R - A')]/[(0{\cdot}5 - A) - (P - A')]$. An examination of the experimental accuracy of the above procedures is presented for the four South African coals, with particular reference to the effects of the purity of the pyridine; it is concluded that the resulting variations in the results are no greater than those obtained by repeated determinations on the same sample, but that the differences obtained by repeated tests on the same sample are so large that many determinations must be made in order to calculate the reactivity index with any degree of certainty. In view of the limitations of the method, Francis (*Fuel*, 1932, **11**, 171; 1933, **12**, 128) and Heathcoat (*id.*, 1933, **12**, 4) have suggested that an alkaline permanganate solution should prove more satisfactory than the acid oxidising agents; 0·5 g. of the extracted sample is therefore ground to pass a 120 I.M.M. sieve but be retained on a 150 I.M.M. sieve, and oxidised by the action of 50 ml. of *N* sodium hydroxide and 200 ml. of *N* potassium permanganate solutions for 1 hour in a boiling water-bath. Then the permanganate number is the number of ml. of *N* potassium permanganate solution utilised by 0·5 g. of ash-free extracted coal; it is expressed in terms of the percentage of ulmins rendered soluble or of the amount of oxidising agent used up. It is concluded that more confidence may be placed in this value, as the experimental error is small, and there is little variation in the results obtained from the same coal by different workers. According to Francis (*loc. cit.*) oxidation of coal by the atmosphere at low temperatures, or by

Hofmeister's reagent, is effectively complete only when the ulmins have been rendered soluble in alkalis, attack being confined to the external groupings of the ulmin molecule. Since, however, alkaline permanganate attacks the nuclear structure also, it provides a measure of the reactivity of coal to a stage further in the direction of complete combustion. The relative merits of the two methods depend, therefore, on the property of the coal which it is desired to investigate. Thus the reactivity index may be expected to correlate well with properties corresponding with the initial stages of combustion (*e.g.* spontaneous combustion or the first stages of destructive distillation), whilst the permanganate number should give a better indication of processes involving the complete breakdown of the molecule (*e.g.* hydrogenation, chlorination and high-temperature distillation). J. G.

Inorganic

Indo-Oxine, a new Precipitant for Metals. R. Berg and E. Becker. (*Z. anal. Chem.*, 1940, **119**, 81–90.)—The reagent is quinolinequinone (5, 8)-[8-hydroxyquinolyl(5)-imide]-5, a reddish-brown crystalline powder of m.p. 253°–254° C., soluble in mineral or glacial acetic acid to form a red solution which changes to green with excess of alkali. In dilute solutions a colour change takes



place at *p*H 6 to 8. Indo-oxine is a serviceable indicator for the titration of very dilute (*e.g.* 0·01 *N*) acid or alkali, but carbon dioxide interferes and must be expelled by boiling. In acetic acid or neutral solutions indo-oxine forms a sparingly soluble bluish-green silver salt, and it can therefore be used as an indicator for the titration of halogens. It also forms blue or bluish-green precipitates with a number of other metals in acetic acid or ammoniacal solution, and is particularly suitable for the micro-determination of copper, nickel and mercury. *Copper.*—A neutral solution, containing from 0·2 to 1·0 mg. of copper, is treated with 5 ml. of 2 *N* acetic acid and 5 ml. of conc. sodium acetate solution, diluted to 50–60 ml. and heated to 60°–70° C., after which it is titrated with an alcoholic 0·05 per cent. solution of indo-oxine. Since the blue colour of the precipitate masks the red colour of the liquid, it is necessary after each addition of the reagent to filter a few drops through a quantitative micro-filter. *Nickel* and *mercury* are determined in the same way, but the mercury solution must be free from halogens, which form soluble complex compounds with the reagent. Thus minute quantities of copper may be titrated in presence of mercury by nearly neutralising the solution (containing 0·2 to 1 mg. of copper and 0·25 to 0·75 g. of mercury), adding 5 ml. of 2 *N* acetic acid, 5 ml. of conc. sodium acetate solution and 5 g. of sodium chloride, heating the mixture to 60°–70° C. and titrating it as described above. W. R. S.

Partial Separation of Copper from Small Amounts of Arsenic. B. Park. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 97–98.)—The sample of copper (10 g.) is dissolved in a mixture of 100 ml. of water, 20 ml. of conc. nitric acid and 20 ml.

of conc. sulphuric acid. Nitrogen oxides are removed by heating, 10 g. of tartaric acid are added, the liquid is diluted to 350 ml., and the bulk of the copper is removed by electrolysis. Arsenic may be determined in the solution by any convenient method. S. G. C.

Stability of Peroxidised Titanium Solutions. G. H. Ayres and E. M. Vienneau. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 96.)—Colorimetric studies showed that the yellow colour developed by titanium and hydrogen peroxide in dilute sulphuric acid remained unchanged for 2 years, indicating great stability of the colour. The use of artificial colour standards is therefore unnecessary. S. G. C.

New Method for the Removal of Nitric Acid. E. Müller and C. Burchard. (*Z. physiol. Chem.*, 1940, **263**, 47–48.)—The removal of large quantities of nitric acid is best accomplished by conversion into barium nitrate, which is sparingly soluble in methyl or ethyl alcohol. Baryta is added to the solution, and carbon dioxide is passed through it to precipitate the excess. After separation of the barium carbonate, the filtrate is concentrated to a small bulk and four volumes of methyl alcohol are added. Most of the barium nitrate is precipitated and is filtered off after standing overnight; the filtrate is then distilled under reduced pressure to remove the alcohol. The rest of the nitric acid is removed by the following procedure, which is also recommended when only small amounts of nitrates are present in a solution:—A solution of 15 g. of nitron in 100 ml. of 5 per cent. sulphuric acid and 500 ml. of water is added until no further precipitate forms. The insoluble nitron nitrate is immediately filtered off, and the filtrate is treated with aqueous picric acid solution, a large excess being avoided. The precipitate of nitron picrate is filtered off, the filtrate is acidified with sulphuric acid, and the picric acid is extracted with ether. After removal of the sulphuric acid, a solution of the free base remains. F. A. R.

Microchemical

Rapid Method for the Accurate Estimation of Minute Quantities of Arsenic in Biological Material. J. Bodnár, Ö. Szép and V. Cieleszky. (*Z. physiol. Chem.*, 1940, **264**, 1–22.)—Gangl and Sánchez (*Z. anal. Chem.*, 1934, **98**, 81; Abst., ANALYST, 1934, **59**, 716) introduced three improvements into the ordinary Marsh test. The first of these consisted in the use of zinc with a large surface-area, the second in the use of a quartz spiral instead of a straight tube for producing the arsenic mirror, and the third in dissolving the arsenic in iodine chloride solution and titrating the liberated iodine with potassium iodate solution after addition of potassium cyanide. Several further refinements have now been incorporated in this method, enabling the estimation of 0·3 to 5γ of arsenic to be made with a high degree of accuracy. The method comprises three separate stages. For the first stage, a 100-ml. conical flask fitted with a stopper carrying two tap-funnels and an air condenser is used. The minced tissue (from 10 to 15 g. according to the arsenic-content), or the dry solids obtained from such tissue or body fluid (2 to 3 g.), are weighed into the flask and thoroughly mixed by stirring

with 1 ml. of 50 per cent. sodium chlorate solution. The flask is then stoppered, and 4 ml. of fuming hydrochloric acid (sp.gr. 1·19) are run into it from one of the tap-funnels. The flask is immersed in a boiling water-bath, and after a few minutes a further ml. of acid is run in with constant shaking, followed by 2 ml., which are added dropwise. When the reaction-mixture begins to froth, the flask is removed from the water-bath, and 1 ml. of 50 per cent. sodium chlorate solution is added from the second funnel, and another 1 ml. of acid simultaneously from the other tap-funnel. After a second period of heating, the decomposition is complete and the solution is allowed to cool and then diluted with a little dilute hydrochloric acid (1 part of conc. acid and 2 parts of water) and warmed to expel residual chlorine. The second stage of the estimation comprises the reduction of the solution and the formation of an arsenic mirror. Spongy tin and hydrochloric acid are used as the reducing agent; unlike zinc and sulphuric acid they do not convert antimony into its hydride. Tervalent arsenic compounds are reduced in the cold, but quinquevalent compounds only on boiling. Consequently, tervalent compounds, if present, must first be oxidised; otherwise arsine may be liberated during the preliminary removal, by hydrogen, of air from the apparatus. Twenty g. of spongy tin* and 6·5 ml. of fuming hydrochloric acid are added to the flask in which the digestion was carried out, and then sufficient water to cover the tin. The flask is closed with a stopper carrying, first, a tube connected (*via* wash-bottles containing potassium permanganate solution and conc. sulphuric acid) with a hydrogen generator and, secondly, a 10-ml. pipette bent at right angles. The other end of the pipette is connected by rubber-tubing with the quartz spiral already referred to (internal diameter 1 mm. at the end connected with the pipette, 0·2 mm. for the rest of the tube), and this in turn is connected with a small tube dipping into water to indicate the rate of flow of the gas. The pipette serves as a condenser and has a length of woollen thread wrapped round it, this being kept moist by a stream of cold water. A similar thread is wrapped round the far end of the quartz spiral where the arsenic mirror is to be formed. The current of hydrogen is turned on and, after about 12 minutes, the spiral is heated with the flame of a fish-tail burner, 8 cm. long and 3 to 4 cm. high. The current of hydrogen is reduced in speed, and the flask is gently heated over an asbestos gauze, the temperature being gradually raised so that boiling begins in 30 to 40 minutes. As hydrogen is evolved by the contents of the flask, the stream of hydrogen is cut off altogether. Boiling is continued for a further 10 minutes, after which both flames are extinguished, and the apparatus is allowed to cool with a current of hydrogen passing through it from the generator. The third stage comprises solution of the arsenic mirror formed on the quartz tube and titration of the arsenic solution with the aid of a micro-burette. Into a 5-ml. titration flask is introduced 0·2 ml. of iodine chloride solution (prepared by dissolving 1·56 g. of potassium iodide and 1 g. of potassium iodate in 50 ml. of water and pouring the solution into 50 ml. of conc. hydrochloric acid. A small

* The spongy tin is prepared by inserting sticks of zinc or aluminium (2 to 4 mm. diameter) in a (diluted) solution of stannous chloride in hydrochloric acid. The precipitated tin is filtered off and thoroughly washed. It is not arsenic-free, however, and is therefore boiled for 1 to 2 hours with conc. hydrochloric acid diluted with an equal quantity of water. The traces of arsenic present are reduced to arsine, which is boiled off, and the undissolved arsenic-free tin is filtered off, washed and dried. The solution can be used for preparing further quantities.

amount of free iodine is removed by adding dilute potassium iodate solution dropwise until a drop of carbon tetrachloride added to the solution is no longer coloured). The narrow end of the quartz tube is immersed in this solution, which is forced in and out of the tube until the mirror is dissolved. The tube is then washed through with 0·06 ml. of iodine chloride solution from a fine pipette, followed by a few drops of dilute hydrochloric acid. Into the flask are then put 0·3 ml. of 10 per cent. potassium cyanide solution and 3 to 4 drops of carbon tetrachloride. The flask is stoppered, vigorously shaken and allowed to stand for 15 minutes. The liberated iodine is titrated with 0·001 *M* potassium iodate solution from a specially designed 0·1-ml. micro-burette graduated in 0·001 ml. The end-point is indicated by the disappearance of colour from the carbon tetrachloride. A blank estimation must be made upon the reagents only, the value obtained being subtracted from the value found for the test solution. The whole operation can be carried out in less than 3 hours, and arsenic added to biological material in amounts varying from 0·3 to 5γ, was estimated with a maximum error of +3·7 or −3·4 per cent. F. A. R.

Micro-Determination of Zinc by the Hydroxyquinoline Method. IV. Separation from Manganese, Iron, Bismuth, Mercury, Arsenic and Antimony. C. Cimerman and P. Wenger. (*Mikrochem.*, 1939, **27**, 76–84.)—I. *Separation from manganese salts.*—A double precipitation is necessary. The solution, containing about 1 to 3 mg. of zinc in 1·5 ml., is treated in a microbeaker of Jena glass with a drop of universal indicator, 2 drops of 10 per cent. acetic acid and a few drops of a 40 per cent. solution of sodium acetate until the *p*H is 5–6. The solution is then heated to boiling, treated with excess (0·1 to 1 ml.) of a freshly prepared 1 per cent. alcoholic solution of 8-hydroxyquinoline, and filtered after 10 to 30 minutes through a filter-stick. The precipitate is washed with double-distilled water and dissolved in 3 ml. of 2 *N* hydrochloric acid and 2 ml. of water. Then 0·5 ml. of 40 per cent. sodium hydroxide solution is added, followed by a 4 per cent. solution until a slight cloudiness appears; this is dissolved in glacial acetic acid, and the solution is treated with about 2 ml. of glacial acetic acid and a solution of 3 g. sodium acetate in water. Water is added to bring the volume to 10 or 20 ml. according to the amount of manganese present. II. *Zinc in presence of iron.*—The solution is treated in a 50-ml. conical flask with 1 ml. of 30 per cent. tartaric acid solution, 2 drops of a 0·1 per cent. alcoholic solution of bromothymol blue and sufficient 8 per cent. sodium hydroxide solution to change the indicator colour. Zinc is precipitated with the reagent at 120° to 130° C. in about 10 ml. of the solution. III. *Zinc in presence of bismuth.*—The precipitation is made in alkaline solution on the same lines as II above. IV. *Zinc in presence of mercury.*—The precipitation is made in alkaline solution as in II, but potassium cyanide is added to prevent precipitation of the mercury 8-hydroxyquinoline compound; as small an excess as possible must be added. V. *Zinc in presence of arsenic and antimony.*—The same procedure is used as in II, the precipitation being made from alkaline solution. The method is the same. It is not possible to effect a microanalytical separation of zinc from nickel or cobalt by the method. A summary of previous papers on the hydroxyquinoline method is appended. J. W. M.

Microchemistry of the Rare Earths. G. Beck. (*Mikrochem.*, 1939, **27**, 47–51.)—*Determination of scandium and thorium.*—Scandium and thorium alone among the rare earths give precipitates with alizarine-3-sulphonate, difficultly soluble in acetic acid. Very small amounts of scandium and thorium may be separated in a single precipitation from the weakly basic fraction of the rare earths. A 2·5 per cent. solution of sodium alizarine-3-sulphonate is used as reagent, with subsequent addition of 30 per cent. acetic acid. The precipitate is very voluminous and unsuitable for filtration with suction, owing to its slimy nature. It is washed with water, centrifuged, and finally dried at 100° C., and is then in a suitable state for conversion into sulphate by treatment with sulphuric acid containing a little nitric acid. *Reactions of rare earths with tincture of cochineal.*—The rare earths and zirconium compounds yield violet solutions with tincture of cochineal, whereas thorium salts give blue solutions. Only lead and copper salts interfere, as they give a blue precipitate with the reagent. Beryllium, aluminium and gallium salts merely give a pink colour with a red fluorescence in ultra-violet light. Red colours are given by zinc, mercury, cadmium, calcium, magnesium, barium, strontium, nickel, cobalt and manganese salts. The violet solutions of the cerite earths from lanthanum to samarium become reddish-orange on the addition of dilute acetic acid, the original colour of the cochineal tincture being restored. With terbium and erbium earths and zirconium, strong acetic acid is necessary to change the colour to red. The concentration limit for the earth metals is 10γ in 1 ml. J. W. M.

Physical Methods, Apparatus, etc.

Luminescence Analysis of Rare Elements. M. Servigne. (*Bull. Soc. Chim.*, 1940, **184**, 121–132.)—Most of the rare earths, when incorporated in a suitable solid, will form phosphors which give characteristic cathodo- or photo-luminescence. At ordinary temperatures the emission spectrum consists of lines or narrow bands capable of precise measurement. The emission spectra of phosphors activated by praseodymium, neodymium, samarium, europium, terbium, dysprosium, holmium, erbium and thulium occur in the visible region, and by gadolinium, lanthanum, and cerium in the ultra-violet; infra-red emission is given by praseodymium, neodymium, samarium, europium and dysprosium. The luminescence of these phosphors is not a simple phenomenon; in addition to the activating element it owes its particular characteristics to several other factors, such as chemical composition of the base material, method of preparation, preliminary heat treatment and final crystalline state of the luminescent solid solution. A further variable is the nature and intensity of the exciting energy. When more than one activator is present, masking effects may occur. The more sensitive activators, Pr, Sa, Eu, can be detected at concentrations of 1 in 100,000. The author considers calcium tungstate to be a suitable base material for activation by rare earths. The calcium tungstate is prepared by heating an intimate mixture of calcium oxide and tungstic oxide at 1100° C. for 2 hours. It should contain less than 1 p.p. 10^5 of iron and should be free from lead, copper and silver. The best conditions for observing the luminescence spectra are obtained when the phosphor is held at 90° C. The author describes two methods depending on

photo-luminescence. In the first, the source of excitation is a quartz mercury discharge tube (7 mm. diam., 0·5 mm. wall thickness), 30 cm. long and consuming 5 watts. A tube of this type gives 65 per cent. of its energy in the U.V. at 3650 Å and 2537 Å; tungstates are particularly sensitive to the latter radiation. The luminescent substance is placed on a quartz sleeve in a small electric furnace surrounding the tube, and observations are made through a small window in the furnace. In the second method, a mercury discharge tube in the shape of a double U is operated with the substance coated on the inside walls of the tube near the electrodes. This method avoids the necessity for a furnace. The luminescence is photographed on suitable plates. In the infra-red, exposures up to 48 hours may be necessary. Limiting concentrations of various rare earths in calcium tungstate, as determined by these methods, are tabulated. For quantitative estimates the intensities of the spectra are compared photometrically with those of prepared standards. It is claimed that samarium and other rare earths can be determined with a precision of the order of 5 per cent. B. S. C.

Spectrovolumetric Determination of Alkaline Earths and Phosphate. T. Török. (*Z. anal. Chem.*, 1940, **119**, 120–125.)—The non-volatility of the phosphates of the alkaline earths in a Bunsen flame (ANALYST, 1939, **64**, 383) can be utilised for the volumetric determination of either alkaline earths or phosphate, the technique described in the abstract referred to above being applied. The strongly acid chloride solution of the alkaline-earth metal, containing some pieces of zinc, is titrated with *N* ammonium dihydrogen phosphate solution until the flame placed horizontally above the solution, viewed through a spectroscope, fails to give the characteristic lines of calcium, strontium, or barium. The method is reasonably accurate, and can be used conversely for the titration of acid solutions of phosphate by means of *N* strontium chloride solution. W. R. S.

Low-Temperature Cooling Baths. H. L. Wikoff, B. R. Cohen and M. J. Grossman. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 92–94.)—The use of mixtures of solid carbon dioxide with organic liquids was investigated. Solid carbon dioxide was added to the liquids contained in vacuum flasks until no further reduction in temperature occurred. The following liquids were considered the most suitable for practical use, as they neither crystallised nor became viscous:

Liquid	Temperature produced °C.
Ethylene glycol	−15
Dimethoxytetraethylene glycol	−31
Diethyl carbitol*	−52
Carbitol acetate*	−67
Cellosolve*	−73
Cellosolve acetate*	
Diacetone alcohol	
Butyl cellosolve*	

When the lowest temperature was required it was found convenient to use a series of low-temperature baths rather than to place the object at air temperature into the coldest mixture, which might result in violent evolution of carbon dioxide. The same fluid may be used repeatedly, more solid carbon dioxide being added as required. S. G. C.

* Trade names.

Reviews

PHYSICO-CHEMICAL METHODS. Vol. I. MEASUREMENT AND MANIPULATIONS. Pp. xv + 686. Vol. II. PRACTICAL MEASUREMENTS. Pp. ix + 580. By JOSEPH REILLY, M.A., D.Sc., F.Inst.P., F.I.C., and WILLIAM NORMAN RAE, M.A., Sc.D., F.I.C. London: Methuen & Co., Third Edition. 1940. Price £4 4s.

All chemists use physical methods to some extent, and such instruments as the polarimeter and microscope are to be found in every laboratory. The spectrograph is taking a prominent place in the larger laboratories, and physico-chemical methods are being utilised more and more, not only for research but also for routine analytical purposes. Some physical instruments may have a relatively small application outside certain specialised fields; but others, *e.g.* the polarograph, have a very much wider scope than might be guessed by those unacquainted with them. It is often complained that physical apparatus is very expensive. This may be true for complete, ready-to-use instruments, housed in polished cases; but when the physico-chemical principles of a method are understood, a "hook-up" will serve, nay, may be preferable, since it can be modified to suit particular conditions. Many physico-chemical processes need no special apparatus other than that which any worker can make for himself.

The practising chemist needs to be able to keep abreast of the progress in allied sciences, to refresh his memory on the principles underlying the methods used, and to consider their adoption for his particular purposes. The work under review is an admirable source for satisfying these needs in respect of physics. Volume I covers the general plan of a laboratory; measuring units; statistical and graphical representation of results; apparatus; manipulative operations; high pressure technique and some particular physical measurements. Volume II includes separations involving distillation, crystallisation, centrifugal and other mechanical processes; optical and electro-chemical measurements; indicators and *p*H measurement; applications of the thermionic vacuum tube; dielectrics and radioactivity. These are only the main headings, for the subject matter is, as the preface says, "an orderly collection of borderland problems wherein both physicists and chemists co-operate." Each volume contains over 400 diagrams.

Again to quote the preface, "the temptation to convert the work into an unwieldy encyclopaedia has been resisted." For this the authors are to be congratulated, since it has not been coupled with a reduction of the number of methods dealt with, but only with the number of pages devoted to each. Some parts may be found too concise for practical purposes; but the facts are there, and many references are given to suitable books and original papers for further reading on the subject matter of each chapter.

In each volume the index occupies parts of two pages only. This has been done deliberately to avoid making it, as a note says, "so detailed as to be clumsy and ineffective." In the reviewer's opinion it is now ineffective because it is so lacking in detail, being of little more value than the table of contents. The

arrangement of the subjects is such, however, that the interested reader will soon find his way about without an index.

No modern chemical laboratory can afford to be without these two volumes. Although the price is high, it must be equated to the comprehensive nature of the subject matter. J. R. NICHOLLS

BELL'S SALE OF FOOD AND DRUGS. Tenth Edition. By R. A. ROBINSON, O.B.E., Barrister-at-Law, Chief Officer, Public Control Department, Middlesex County Council, and ROBERT IVES, Barrister-at-Law. Pp. xxvii + 363. London: Butterworth & Co. (Publishers), Ltd., and Shaw & Sons, Ltd. 1939. Price 15s. net.

"This little volume is intended to supply a want which it is believed has been felt by persons who are engaged in carrying out the Sale of Food and Drugs Acts. Great care has been taken to include all cases bearing upon the working of the Acts, and also those in which disputed questions of a chemical nature have arisen. It is hoped that the book may be found useful not only to those upon whom the working of the Act devolves, but also to the trade and general public." These words written in the preface to the first edition of "Bell's Sale of Food and Drugs" seem to have been almost prophetic, and the hope expressed so long ago as 1886 has been more than well fulfilled.

The issue of a new edition, the tenth, has been made necessary by the enactment of the Food and Drugs Act, 1938, which came into force on October 1st last. This edition has had to be considerably enlarged, as the new Act includes sections relating to a number of subjects which were previously to be found in the Public Health Acts, such as unsound food, food poisoning, the sale of horse-flesh and the cleansing of shell fish. Even with this enlargement, it has been possible still to retain such statutes as the Merchandise Marks Act, 1926, and the Sale of Food (Weights and Measures) Act, 1926, as in the eighth and ninth editions, and to include the Agricultural Produce (Grading and Marking) Acts, 1928 and 1931.

Mr. Robinson, who has been responsible for the preparation of five earlier editions, has now introduced Mr. Ives, also a barrister-at-law, as a co-editor of this authoritative work. The bulk of the present edition comprises the Food and Drugs Act, 1938, carefully annotated, together with the text of the other Acts cited above. A table of replacements shows what provisions have been repealed, where they may be found in *Halsbury's Statutes*, and the corresponding sections of the previous Food and Drugs Acts. Part II deals with Departmental Regulations and Orders.

In this edition 42 cases previously reported in the ninth edition have been omitted and 38 fresh cases inserted. The chemical notes have been augmented with new data, including such references as a suggested maximum of 40 per cent. of water in processed cheese and the inclusion of cooking fats with lard. The possible presence of ailanthus in mint is mentioned, and under the heading Port and Madeira wine there are references to two High Court cases—Holmes *v.* Pipers Ltd. (1914) and Sandeman *v.* Gold (1924). A paragraph on whisky is also included.

Regarding meat, apparently an experienced inspector is expected to identify different kinds of liver simply by making a cursory glance without reference to the

Public Analyst. Mr. Robinson's views on a possible waste of public money on analyses may be observed on page 5, and no doubt the Public Analyst will await with interest the case in which the vendor, having admitted a sale of margarine as butter, declares in Court that it was butter after all. Perhaps the Public Analyst would be permitted to smile, as he certainly might do, at Mr. Robinson's belated discovery that methods other than chemical may be employed.

Little criticism is needed for a book that has reached its tenth edition, and it is pleasant to note that such mistakes as were made in the ninth edition appear now to be corrected. It is safe to say that both Public Analysts and lawyers engaged in work dealing with Food and Drugs certainly owe a debt of gratitude to the two co-editors for bringing this invaluable book up to date.

The publishers propose to issue, from time to time, supplements to this work, and a pocket inside the back cover is provided to hold them.

ERIC VOELCKER

A HISTORY OF CHEMISTRY. By F. J. MOORE, Ph.D. Revised by William T. HALL. International Chemical Series. JAMES F. NORRIS, Ph.D., Consulting Editor. Third Edition. Pp. 429. Index, pp. 47. London: McGraw-Hill Publishing Co., Ltd. 1939. Price 20s.

In common with similar histories this volume devotes its early pages to a description of the events preceding the birth of chemistry as a specific entity. The science of chemistry evolved gradually from the practice of art as applied to metallurgy; its progenitors in the investigation of natural substances included philosophy, astrology, necromancy, religion and chicanery, and its main aims were the search for the elixir of life and the philosopher's stone—tempting baits for men who sought wealth and notoriety. Such chemical knowledge as there was up to the fifteenth century was termed alchemy.

The author has dealt with this part of the subject lucidly and in interesting chapters which lead to the *renaissance* period, when Paracelsus, Agricola, Valerius Cordus, Basil Valentine, Van Helmont and Johann Glauber appear in a description of their work and influence on the subject before Boyle, Mayow and Stahl became prominent and the Phlogiston Theory held the stage for so many years.

This period was the immediate forerunner of the anti-phlogiston era, when Black, Cavendish, Scheele and Priestley made their all-important discoveries, and the recognition of oxygen as an element may be considered to mark the time when more modern ideas were needed to fulfil all the precepts which led us to the fundamental principles of eighteenth-century chemistry.

From this period the author takes his readers consecutively through the work done by Lavoisier and Berthollet to the time of Dalton and the Atomic Theory, and here he shows how the physical properties of matter became an integral part of the study of chemistry. Galvani, Volta and Humphrey Davy made such alterations in the existing ideas and converted them into conceptions so novel that one is hardly surprised at the developments which followed. Organic Chemistry, and all that it foreshadowed, was a new sphere of chemistry from which, during the past century, the whole of mankind has benefitted.

The chapter dealing with Organic Chemistry since 1860 is full of interest. The scientists' names are too numerous to quote, but every chemist is familiar with them in connection with reactions or processes or principles. Many of them are living no longer, but it is of interest to read of the modern work of Haworth on sugars, that of Hans Fischer on physiological chemistry, and that of Karrer on the vitamins. The final chapter in the book is a lengthy one of a hundred pages, so full of the progress of Inorganic Chemistry since 1860 that it deserves a volume apart.

The volume is fully illustrated, and very many of the portraits will be familiar to most English readers. Such references and dates as could be checked are correct, and the volume should be indispensable to students of the subject; it is full of profoundly interesting material for the modern chemist, be he specialist in the organic, inorganic or physical department of the science. C. EDWARD SAGE

AN INTRODUCTION TO BIOCHEMISTRY. By Prof. W. R. FEARON, M.A., Sc.D., M.B., F.I.C. Second Edition. Pp. xii + 475. London: William Heinemann (Medical Books), Ltd. 1940. Price 17s. 6d. net.

It is almost exactly six years since the first edition of Professor Fearon's gallant book was reviewed in this Journal (ANALYST, **1934**, **59**, 372). Much biochemical water has flowed under the bridges of science since that time—and Professor Fearon has faced with renewed gallantry the incorporation in his second edition of all relevant new knowledge gained in the intervening period, though this involved rewriting three-quarters of the book and increasing its length by more than one-half. The price has increased about proportionately—less than might have been expected in present circumstances.

There are very many text-books of biochemistry written in English (more or less), but curiously few written and published in Great Britain and Eire. It is not an exaggeration to say that this one, with the additions, improvements and corrections made since the first edition, is now the best of the home products and a dangerous competitor to the best of the foreign—Bodansky's "Introduction to Physiological Chemistry" (see ANALYST, 1938, **63**, 921).

A work of this kind, having been brought as nearly up-to-date as exigencies of publication permit, is unlikely to be free from undetected slips of phraseology and expression, or to have avoided minor inaccuracies in a few places; still less is it likely that every statement of opinion in it will find general acceptance among biochemists. Indeed, if it did, it is probable that its author might feel that he had in some manner betrayed his Irish nationality. A review is no place for calling attention to these minutiae, except in a general way; individually they are better brought to the author's attention by personal correspondence, especially to an author like Dr. Fearon, who has wisely shown himself unusually willing to accept and act on the friendly criticisms of his many favourable reviewers.

It is, however, necessary for a reviewer to show that he has done something more than "break the back of the book"—which Mr. Belloc's "Caliban" advised as the only essential part of book reviewing. I note that Dr. Fearon uses D- and L- to denote the structural configurations of compounds in relation to their positively and negatively rotating chemical ancestors, reserving the conventional

d- and *l*- to express actual rotation. It is a little difficult to see what advantage this has over the use of *d*- and *l*- for structure and of (+) and (−) for rotation. Moreover, when it comes, for example, to the amino-acids, Dr. Fearon avoids the problem altogether by not crediting them with any optical activity at all, although he has a general statement on the subject early in Chapter IX. What the student needs to know is *both* the configuration of the amino-acids (where this has been established) *and* the directions of their optical rotations. I would suggest that, when revising this book, the author should include in his discussion of fat absorption some consideration of the work of Frazer and Stewart; the implications of this are so disturbing to current complacency about complete intestinal lipolysis that they deserve study for that reason alone—apart from the fact that Frazer's views on the subject are almost certainly correct. One other comment—also on a point of detail. Surely Dr. Fearon is wrong in attributing to Karrer (by implication) the view that "condensation of two phytol residues would in theory yield lycopene" (p. 204). The equation for this reaction would be

$$2C_{20}H_{40}O(1F) = C_{40}H_{78}(2F) + 2H_2O.$$

But the formula for lycopene is $C_{40}H_{56}(13F)$; the condensed molecule needs 11 dehydrogenations before it can have a carotenoid structure.

Leaving lipoids, I can find points of dissent about water-soluble substances, also. The most usual biological test for vitamin B_1 (aneurin) is by rat-growth (weight increase), and is the only practical one *not* mentioned by Dr. Fearon. Again, the author appears completely to accept Kögl's claims to have found optically abnormal isomers among the amino-acids of tumour tissues, although the matter is being hotly contested by experts in protein chemistry, and must at the least be held still to be *sub judice*.

The book contains a certain number of directions for actual chemical tests, doubtless for convenience where it is used as a class text-book, but their number is relatively small and it might be better either to omit them altogether—for the general biochemical user they are an embarrassment rather than a convenience—or to relegate them to an appendix.

I know of no book more suitable to the analyst—or any non-biochemist—who wishes to revive his knowledge (or create it) of modern biochemistry, and of none more handy for the biochemist as a working book of reference, in which respect it sets a very high standard for completeness, lucidity and accuracy.

A. L. BACHARACH

FOOD VALUES AT A GLANCE. By V. G. PLIMMER. Pp. 190. London: Longmans, Green & Co. 1939. Price 6s. net.

Anyone who has ever tried to set out the elements of nutrition in a way sufficiently simple to be understood by non-scientific readers knows the difficulty of the task. The degree to which Mrs. Plimmer has succeeded is proved by the publication of this second edition of her short book. The essential feature of the volume, from which it derives its title, is a series of 26 coloured charts which show graphically the composition of 175 common foodstuffs with respect to protein, fat, carbohydrate, mineral salts, moisture and physiologically unavailable material,

and also indicate the proportion of vitamins A, D, B_1, B_2 and C. It is unquestionable that to these clear and brightly coloured diagrams the book owes a large measure of its popularity. Besides these there are a number of black and white diagrams illustrating such aspects of dietetics as the calorie requirement at different ages, the comparative protein value of food in shillingsworths (with, alas, corned beef well in the lead) and many others. Six charts, new to this edition, compare the amounts of vitamins in different foods.

In the text, the chapters dealing with the elements of nutrition are also clear and easily understood. The rôle of each food constituent is described in turn, with the amount of each needed in the diet. The typical daily bill of fare, for a family or school or institution, comprising these nutritional factors is then worked out. All this is excellent. But a text-book, however small and however "popular," must jealously guard against the intrusion of opinion or the most innocent of generous enthusiasms into a realm where facts alone should rule. Perhaps, for example, appendicitis, colitis and cancer may be due to other causes than "the consumption over many years of vitamin-poor foodstuffs."

While the greater part of the book is derived from the first edition brought up to date, certain additions have been made. The inclusion of an index can be given unreserved welcome. Three additional chapters at the end of the volume, however, represent in its most acute form that slide from the scientific world into the political arena which has already been deprecated in a work of this nature. We can sympathise with Mrs. Plimmer for disliking the agricultural policy of the Government; for disapproving of the business principles under which food is distributed in a capitalist England; for condemning the tipping of town sewage into the sea; but do these views enhance the value of an elementary primer on food values?

From the point of view of the analyst the book has many useful features. The charts briefly summarise the composition of foods and can be a quick aid to memory. The tables of daily human requirements, ranging from calories to vitamins, are also valuable for rapid reference. Though it is strange to see pork demonstrated in the chart as containing no vitamin B_1, while ham, bacon and pork-pie possess it, inaccuracies appear to be few.

"There is probably no field of human thought in which sentiment and prejudice take the place of sound judgment and logical thinking so completely as in dietetics," quotes the author. As they put down the book certain analysts of our acquaintance may sigh. Alcoholic drinks are a common cause of obesity; continental cooking may lead to avitaminoses; we know that tinned salmon, brown bread and cabbage would make a perfect diet—but, oh, how we wish they would not.

MAGNUS PYKE

MANUEL DU SAVONNIER. By A. MATAGRIN. Pp. xvii + 268. Paris: Gauthier-Villars. 1938. Price 30 fr.

This book contains no preface, and there is no indication of the class of reader for whom it is intended. It opens with a very interesting chapter dealing with the early history of soap-making, and with the economic position of the industry in the principal countries of Europe, but the book, as a whole, rather gives the impression

of being written by an author without any very intimate knowledge either of soap-making or of its chemistry.

So far as the description of actual soap manufacture is concerned, the book follows conventional lines and deals, rather superficially, with the preparation of most types of soap, including hard, soft and liquid, household, textile, toilet, and medicated soaps. It may be remarked that 20 hours for the pasting stage (p. 63) appears an extraordinarily long time to allow for this operation. Modern developments, such as attempts to make the saponification process continuous and the use of ethanolamines and of antioxygens, are included. The plant used in the various operations is fairly fully illustrated, but, although the figures are mainly of machinery produced by one of the best-known French soap engineers, they are rather poorly reproduced.

The treatment of the theory of detergency is most inadequate, and the book concludes with a section, of seven pages, on the analysis of oils and soaps, which is so unreliable as to be worse than useless. Thus, the strengths of semi- and decinormal solutions of potassium hydroxide are given as 112 g. per litre and 56 per cent. respectively, the acid value is said to be the Koettstorfer value, and the unsaponifiable matter in a fat is to be obtained by determining the fatty acids after saponification and subtracting the percentage of these from 100. In the analysis of soap no distinction is made between unsaponified fat and unsaponifiable matter, nor between caustic alkali or other free alkali, the free alkali being obtained by salting out the soap, filtering, and titrating the alkali in the filtrate.

W. H. SIMMONS

Publications Received

KINGZETT'S CHEMICAL ENCYCLOPAEDIA. 6th Ed. Revised and Edited by R. K. STRONG. With Foreword by Sir GILBERT MORGAN. Pp. x + 1088. London: Baillière, Tindall & Cox. 1940. Price 45s.

CHEMICALS OF COMMERCE. By E. D. and C. T. SNELL. Pp. viii + 542. London: Chapman & Hall. 1940. Price 28s. net.

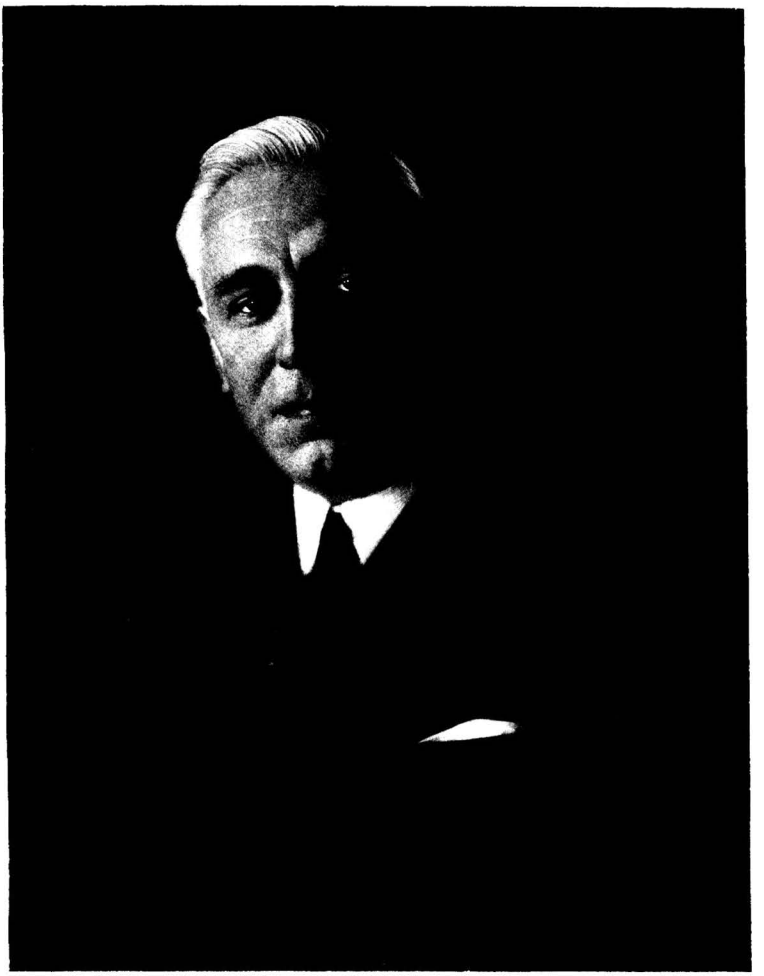
COLLOID CHEMISTRY. By H. B. WEISER. Pp. viii + 428. London: Chapman & Hall. 1939. Price 24s. net.

LES PARFUMS NATURELS. By Y. R. NAVES and G. MAZUYER. Pp. xvi + 398. Paris: Gauthier-Villars. 1939. Price 120 fr.

POISONS: THEIR ISOLATION AND IDENTIFICATION. By F. BAMFORD. Pp. viii+344. London: Churchill. 1940. Price 18s.

A TEXT BOOK ON LIGHT. By A. W. BARTON. Pp. x + 426. London: Longmans, Green & Co., Ltd. 1939. Price 8s.

ANALISI DEI METALLI NON FERROSI. By I. COMPAGNO. Pp. xii + 493. Milan: Ulrico Hoepli. 1939. Price Lire 60.



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