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Obituary

SIR HERBERT JACKSON

SIR HERBERT JACKSON died, after a short illness, at Parsifal Road, Hampstead, on December 10th, 1936, in his 74th year.   He was educated at King's College School, and in 1879 entered King's College, London, where he studied chemistry under Charles Loudon Bloxam, and later under John Millar Thomson, who succeeded Bloxam as Daniell Professor in 1887.   After working for some years as Daniell Scholar and student-demonstrator with Bloxam, he was appointed lecturer and, in 1902, assistant professor.   In 1905 he was appointed professor of organic chemistry and, in 1914, succeeded Thomson as Daniell Professor of Chemistry.   In 1918 he retired with the title of Emeritus Professor, and accepted the appointment of Director of the British Scientific Instrument Research Association, which position he held until 1933.   Subsequently he acted as consultant to the Association and served as a member of its Research Committee.

It will be seen that he was connected with King's College for nearly forty years, in the course of which many who later became members of this Society—including P. A. Ellis Richards, E. R. Bolton, Edward Hinks, F. W. F. Arnaud and John Evans (Past Presidents), and C. A. Mitchell (Editor of THE ANALYST)—came under his tuition.   He took an active interest in all the affairs of the College, and participated in the annual sports, either as a competitor, starter or judge, or in some other official capacity.

He was an attractive lecturer, and although often discursive and unable to resist side issues, he could always pick up the thread again and hold his audience well with experimental demonstrations, which afforded him and them obvious satisfaction, and scarcely, if ever, failed.   He was remarkably skilful in the handling of apparatus and instruments of all kinds, in glass-blowing and in the laboratory arts generally.

In 1883 he contributed to the Chemical Society a note on "The Action of Sulphuric Acid (sp.gr. 1·84) upon Potassium Iodide" and a note on "Bromine as

a Test for Strychnine"; in 1884, a note on the "Action of Arsenious Anhydride on Glycerol."

A writer in *Nature*, January 2nd, 1937, recalls that before 1895

"He carried out a lengthy investigation into the production of phosphorescent materials, and made an extensive study of the phosphorescent and fluorescent phenomena produced in various materials by ultra-violet light or by electric discharge in low-vacuum and high-vacuum discharge tubes. In the course of his experiments with high-vacuum tubes he observed that in some instances phosphorescent materials in close proximity to the tubes gave a luminous response when the tubes were excited. This observation was not followed up immediately, as it did not fall into the general line of investigation which was being pursued at the time, otherwise the discovery of X-rays, which was announced by Röntgen a few months later, might well have been made by (Sir) Herbert Jackson. Immediately Röntgen's discovery was published, (Sir) Herbert Jackson constructed an X-ray tube of the type described by Röntgen—the first X-ray tube to be produced in Great Britain— and thereafter he devoted a considerable amount of attention to a study of the effects which could be produced by the 'new' rays and to the development of X-ray tubes of improved forms. The 'Jackson' focus-tube, which he devised—and which he refused to patent—was a development of very great importance, since it provided practically a point source of X-rays and thus ensured sharp definition in X-ray photographs; the curved cathode which was its essential feature was adopted universally."

The use of the tube was demonstrated at a conversazione held at the Royal Society in May, 1896. On March 5th, 1896, however, he had read a paper before the Chemical Society in which he referred to the use of a cathode tube containing a concave aluminium cathode and a platinum anode inclined at an angle of 45°, *i.e.* the basic type of X-ray tube, unchanged in principle at the present day, which he first used as early as January, 1894 (*Proc. Chem. Soc.*, No. 162, 57–60). Röntgen made his announcement of the discovery of X-rays in December, 1895. Sir Herbert's work was on fluorescence and he had made his tube to deliver invisible radiation outside the tube, in order to study the effect of causation of phosphorescence in minerals and chemicals.

At another time he was interested in the protection of stone-work against weathering, and was consulted with regard to the restoration of Westminster Abbey and Canterbury Cathedral.

In 1898 he gave a discourse on phosphorescence before the British Association at Bristol (*Nature*, **58**), and in 1900 dealt with the same subject in a series of juvenile lectures at the Royal Society of Arts (1900, **48**).

In 1905, jointly with D. Northall-Laurie, he contributed to the Chemical Society a paper on "The Action of Carbon Monoxide on Ammonia," and in 1906, papers on "The Behaviour of Vapours of Methyl Alcohol and Acetaldehyde with Electric Discharges of High Frequency," and on "The Behaviour of Acetylene with Electrical Discharges of High Frequency."

In 1906 he gave a course of juvenile lectures on Combustion and Flame at the

Royal Society of Arts (1906, **54**). About this time he turned his attention to the detergent properties of soaps and solvents and their effect on textiles, gave a series of lectures to launderers in London, to the substantial benefit of their industry, and a series of Cantor Lectures on Detergents and Bleaching Agents used in Laundry Work, before the Royal Society of Arts (1907, **55**).

In his investigation of glasses, for the Research Committee of the Institute of Chemistry and subsequently for the Ministry of Munitions, which he started shortly after the outbreak of war and continued until the armistice, he found his métier and his great opportunity. He was indefatigable and, in spite of difficulties arising from the shortage of supplies of certain essential materials, produced in a very little time—a few months—more than 70 formulae for batch mixtures for the manufacture of glasses and enamels, including optical glass, glass for miners' lamps, glasses for combustion tubing, capillary and thermometer tubing, laboratory apparatus, X-ray tubes, and ampoules, opal glass and enamels for artificial eyes, sealing-in enamels and vitrite for electric lamps, almost all of which had previously been imported from abroad. A glass similar to his miners' lamp glass was immediately adopted for cooking vessels which are now in general use. His early formulae were published in the Proceedings of the Institute of Chemistry, April, 1916.

Sir James Dobbie, then President of the Institute, gave him full credit for his remarkable achievements and, in March, 1916, the President of the Board of Trade expressed the Board's appreciation of the services rendered by Professor Jackson to the glass industry. In 1919 he delivered the Sir Henry Trueman Wood lecture at the Royal Society of Arts, on Glass and some of its Problems (1920, **68**).

He was elected a member of the Royal Institution in 1924, served as a Manager from 1930 to 1932 and 1934 to 1935. In March, 1927, he gave a discourse before the Institution on "Some Colouring Agents in Glasses and Glazes."

He demonstrated that it was possible to grow green plants in an atmosphere devoid of carbon dioxide, but containing from one to seventy per cent. of carbon monoxide. He also confirmed the formation of starch during such growth. Further work in this connection was carried out by Professor Bottomley and (Sir) Herbert Jackson at King's College, and the results were communicated to the Royal Society (**72**, **130**).

He published very little else, but he did a great deal of work of which there is no permanent record.

If he did not achieve the reputation of a distinguished chemist so soon as he deserved, it was due to his dislike of putting pen to paper, although he was ever ready to impart his findings to his colleagues and students. Indeed, fellow chemists and others were often indebted to his inventive genius for original ideas, in the solution of problems and in devising chemical and physical apparatus, for which he received no material advantage and little or no credit.

Those who worked with him have remarked that, at times, he seemed to possess imagination or intuition which enabled him to foretell results with almost uncanny accuracy. Indeed, when a research worker suggested a problem he would very likely be informed by Professor Jackson that he himself had already worked on it and had obtained certain results. This was a little disconcerting,

perhaps, and it was sometimes thought that he had deceived himself, or was merely surmising, but he was invariably right as to the results.

His other active work for the Institute of Chemistry, in addition to training many candidates for the Associateship, included an Examinership for the Intermediate Examination in general chemistry, which he held from 1908 to 1912, and in Mineral Chemistry for the final examination for the Fellowship, from 1911 to 1915. He was a Member of the Council from 1904 to 1907; a Vice-President from 1907 to 1908, 1916 to 1918, and 1921 to 1924; and a Censor from 1918 to 1935.

As President of the Institute (1918–1921) he was a good chairman, considerate to any opposition, clever in debate and definite in his decisions. Shortly after his election to that office, the Council revised the general policy of the Institute, so that he was called upon to guide its affairs during a period of unusual difficulty and responsibility, a period of reconstruction and resettlement following immediately on the termination of the war.

He was President of the Röntgen Society from 1901 to 1903, served on the Council of the Chemical Society, was a Vice-President and Member of Council of the Royal Society of Arts, an Honorary Fellow of the Royal Microscopical Society, Chairman or Member of several Committees connected with the Department of Scientific and Industrial Research and, for several years, a Member of the Senate of the University of London. He was elected a Fellow of King's College in 1907 and a Fellow of the Royal Society in 1917, and received the Honour of Knight Commander of the Most Excellent Order of the British Empire in the same year.

Of a happy disposition, versatile and entertaining, he will long be remembered by a host of old students and friends as a kind-hearted and public-spirited man.

He was elected a member of this Society in 1915.

RICHARD B. PILCHER

The Enzymes of Milk

I. Some Notes on Kay and Graham's Phosphatase Test*

By E. B. ANDERSON, M.Sc., F.I.C., Z. HERSCHDÖRFER, Ph.D., AND
F. K. NEAVE, B.S.A.

(Read at the Meeting, December 2, 1936)

THE legal definition for pasteurisation of milk laid down for Great Britain has been reiterated in the new Milk (Special Designations) Order, 1936, which came into operation on 1st June, 1936. Apart from the Orla–Jensen Creamometric test,[1] which has certain limitations and which has never been adopted to any

extent in this country, the controller of a pasteurising plant has possessed in the past no test of even approximate accuracy by which he could satisfy himself whether or not a sample of milk had been correctly treated.   The need is clear therefore for a sensitive test to distinguish between milks heated correctly and those heated at a lower temperature than 145° F. or for a shorter time than 30 minutes.

Kay and Graham[2] have described a method based on the thermolability of the enzyme phosphatase in milk, which appears to meet all reasonable requirements for such a test.   Their method has been recommended by the Ministry of Health[3] for use by local authorities.   For details of the test reference should be made to the original paper.

We have subjected Kay and Graham's technique (their "Test B") to a careful examination, using the Zeiss–Pulfrich photometer for most of the colour determinations made.

REAGENTS AND APPARATUS.

STABILITY OF REAGENTS.—(i) *Buffer substrate.*—It has been found that if the buffer substrate solution of disodium phenyl phosphate and sodium veronal is kept in a refrigerator no hydrolysis appears to occur during at least 9 weeks. Even after 24 hours at 37° C., disodium phenyl phosphate does not show any evidence of hydrolysis at pH 9·8 (see Table I).

TABLE I

BUFFER CONTROLS TEST

Buffer sample	Extinction coefficient k	Lovibond blue units	Remarks
1	0·0806	0·3	Freshly prepared
,,	0·097	0·3	After 24 hours' incubation at 37° C.
2	0·075	0·3	Freshly prepared
,,	0·079	0·3	After 9 weeks in refrigerator

(ii) *Folin's phenol reagent.*—If correctly prepared the Folin reagent will be entirely free from any greenish tinge.   We found it an advantage first to allow the bromine to act at room temperature for two hours and then to boil off the excess.   The reagent did not show any sign of decomposition in four months.

The diluted Folin reagent (1 in 3) undergoes no change over a period of one week.

(iii) *Sodium carbonate solution.*—It has been found that, even when using AnalaR sodium carbonate, a precipitate formed in the solution after a short time.

As the intensity of the colour obtained by the test depends to a large extent on the concentration of this solution, it is advisable to control the strength by titration.   It is also advisable to add a few drops of chloroform to the solution.

PRECAUTIONS AND SUGGESTIONS REGARDING APPARATUS.—(i) *Pipettes.*— Any errors in the measurement of the 0·5 ml. of milk will greatly affect the result; therefore only Grade A N.P.L. pipettes should be used.

A separate clean pipette must be used for each sample to be tested, as an admixture of as little as 0·1 per cent. of raw milk with a properly pasteurised milk

may be sufficient to give a value above 2·3 Lovibond blue units. Accurate pipettes should also be used for delivering 2·0 ml. of sodium carbonate to each tube.

(ii) *Test tubes.*—Test tubes marked at 10 ml. should be used to receive the first filtrate, the maximum error tolerated being ±0·1 ml. Tubes on the market often show more than this degree of error, and should therefore be tested before use.

(iii) *Filter-papers.*—Whatman No. 30 filter-papers have been used as recommended, but No. 42 (9 cm.) papers give more satisfactory results.

(iv) *Cleaning of glassware.*—All glass apparatus, including sample bottles, should be washed with hot soda solution and rinsed thoroughly with distilled water. The use of a phenol soap must be strictly avoided.

(v) *Apparatus for colour determination.*—Accurate readings may be obtained with a colorimeter or with a photometer. With the latter an accuracy of 0·02 unit is possible. Whilst such accuracy is not necessary in routine tests, it may be of value in precise investigations on phosphatase activity.

For making standard solutions for colour comparison in a colorimeter, etc., it has been recommended that dilute phenol and Folin's reagent should be used. We have found it equally satisfactory to use a solution of standard strength of a mixture of permanent dyestuffs. For this purpose, 0·10 g. of chlorazol brown L.F.S., 0·20 g. of chlorazol fast grey 2 G.S., and 0·30 g. of chlorazol steel blue 6 B.S. are dissolved together in 1 litre of water.* For use, 10 ml. of this stock solution are diluted with 28 ml. of distilled water; the liquid obtained gives 2·3 Lovibond blue units when tested in the tintometer in standard 13 mm. (rectangular) tubes.
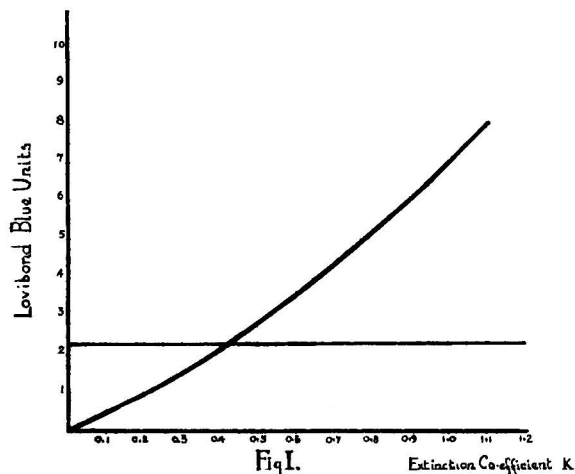

Fig I.

Extinction Co-efficient K

Fig. 1 shows the relation between Lovibond blue units and the extinction coefficient with a selective red light filter, in the range of $750m\mu$. The agreement is surprisingly good.

As is hinted above, the colour produced under the conditions of the test is not a true blue, but contains some red and yellow. It has been proved, however, that the amount of red and yellow is proportional to the amount of blue. The colour is stable for at least three days.

It should be mentioned that the colour is not specific for phenol, but is given by other substances such as ferrous iron and tin salts, tyrosine, etc. These substances, which should not be present in sufficient quantity in dairy products to be detectable by Kay and Graham's test, would in any case indicate their presence in the controls, and therefore would not be mistaken for enzyme-produced phenol.

SAMPLING OF MILK.—The usual care should be exercised in taking representative samples.

* Our thanks are due to Imperial Chemical Industries, Limited, who kindly supplied suitable dyestuffs.

Milk samples taken from the refrigerator should be warmed to 15–20° C. before testing, and must be thoroughly shaken to ensure adequate mixing of the cream. This is particularly important when a cream plug has formed, as the major portion of the phosphatase appears to be associated with the fat-globules. The 0·5 ml. of milk used for the test should be taken from the lower half of the sample bottle and the pipette wiped clean before delivery of the contents.

EFFECT OF VARIATIONS IN TECHNIQUE.—(i) *Incubation.*—For incubation a water-jacketed incubator, or preferably a water-bath, either of which should be controlled at 37° C. ± 0·5° C., is essential. Table II shows the influence of different periods of incubation.

TABLE II

Ref. No.	Time of incubation Hours	Extinction coefficient k	Lovibond blue units
902	24	0·404	2·2
,,	26	0·425	2·3
904	24	0·490	2·7
,,	48	0·593	3·6
567	23	—	2·7
,,	24	—	2·7
,,	25	—	2·7

Variations of half an hour in the period of incubation do not seem to affect the result.

(ii) *Cooling.*—After incubation the tubes should be cooled below 20° C. Table III shows the effect, on the colour, of inadequate cooling before the Folin reagent is added.

TABLE III

Ref. No.	Temperature when the Folin reagent is added °C.	Extinction coefficient k	Lovibond blue units
903	5	0·418	2·2
,,	37	0·431	2·3
904a	5	0·490	2·7
,,	37	0·508	2·8
1104	20	0·360	1·9
,,	37	0·385	2·0

(iii) *Folin's reagent.*—In Table IV is shown the effect of varying the quantity of Folin's reagent added. It is evident that variations likely to occur have no effect.

(iv) *Filtration.*—Table V shows the effect of delay in filtration and emphasises the necessity of close adherence to the correct time, *viz.* 3 minutes, between adding the Folin's reagent and filtration.

A useful procedure is to collect the filtrates in test tubes marked at exactly 10 ml., and to remove the funnels as soon as 10 ml. of filtrate are obtained. This procedure, which avoids the necessity for using a separate 10-ml. pipette for each sample, has also the advantage of being an automatic time control.

TABLE IV

Folin's reagent	Lovibond blue units					
	Milk heated at 145° F. for 30 minutes			Milk heated at 142·5° F. for 30 minutes		
*2·0 ml.	2·1	2·2	2·2			
*2·5 ml.	1·7	2·0	2·0			
*3·0 ml.	1·9	2·0	2·0			
3·5 ml.	2·0	2·0	2·1			
4·0 ml.	2·0	2·1	2·1	5·0	5·0	5·0
4·5 ml.	2·0	2·1	2·1	5·0	5·0	5·1
5·0 ml.	2·1	2·1	2·2	5·0	5·0	5·0
5·5 ml.	2·2	2·3	2·3			

* Contains a high proportion of yellow, and blue colour has a tendency to settle out.

(v) *Addition of sodium carbonate.*—Fig. 2 shows the influence of different quantities of sodium carbonate added and demonstrates the necessity for precise measurement.

TABLE V

Ref. No.	Time of standing before filtration Minutes	Extinction coefficient k	Lovibond blue units
902	3	0·404	2·2
,,	15	0·478	2·7
904b	3	0·490	2·7
,,	15	0·504	2·9
1104a	3	0·360	1·9
,,	30	0·446	2·4

(vi) *Concentration.*—Fig. 3 shows the influence of changes in the concentration of sodium carbonate solution, 2 ml. of solution per test being used.

(vii) *Boiling.*—The effect of delayed boiling after addition of sodium carbonate solution is shown in Table VI.

TABLE VI

Treatment prior to boiling	Lovibond blue units				
	Sample 1			Sample 2	
Boiled immediately and without shaking	4·7	4·6	4·9	5·4	5·4
Mixed, then boiled after 1 min.	4·4	4·4	4·4	5·1	5·1
Mixed, then boiled after 5 mins.	4·3	4·3	4·3	5·0	5·0
Mixed, then boiled after 10 mins.	4·2	4·2	4·2	4·9	4·9
Mixed, then boiled after 30 mins.	4·1	4·1	4·1		
Mixed, then boiled after 40 mins.	4·2	4·2	4·2		

The effect of prolonged boiling on the development of blue colour is shown in Table VII.

TABLE VII

	Lovibond blue units				
Placed in boiling water for 5 minutes	4·2	4·2	4·3	4·3	
Boiled for 5 minutes	4·3	4·3	4·3	4·3	4·3
Boiled for 10 minutes	4·2	4·2	4·1	4·1	4·1
Boiled for 15 minutes	4·2	4·1	4·1	4·1	4·1
Boiled for 25 minutes	4·2	4·2	4·1	4·1	

The test should be made in duplicate and the results should agree within 0·1 Lovibond blue unit.
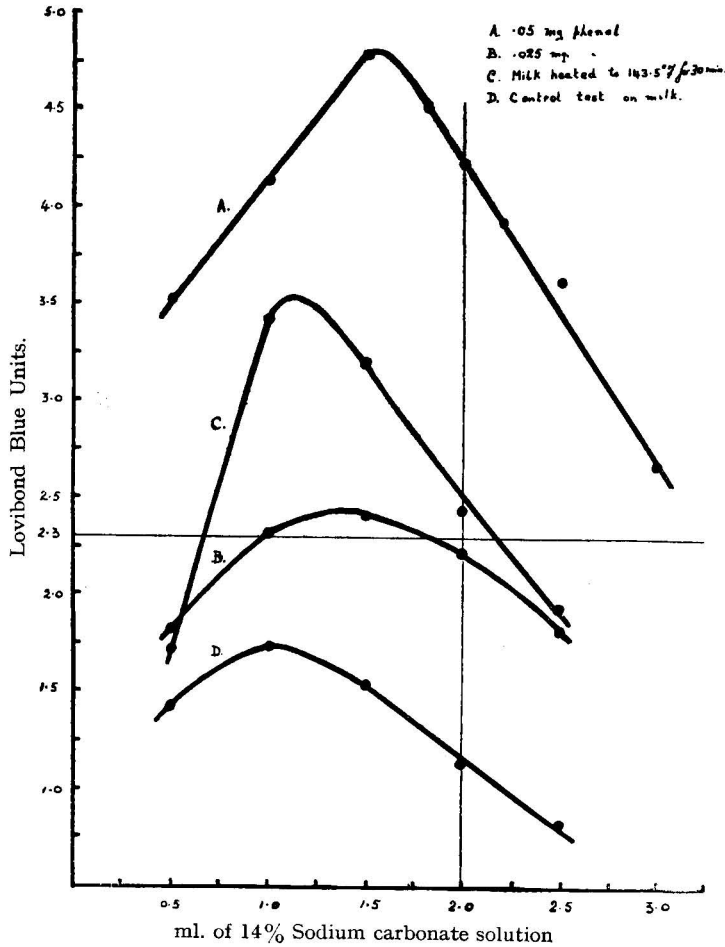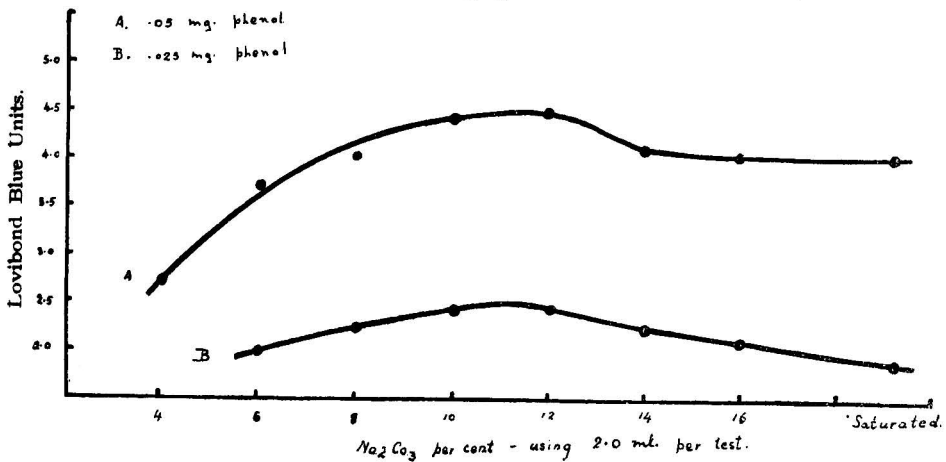

FIG. 2.

FIG. 3.

CONTROL TESTS.—The control tests, in which the activity of the phosphatase has been inhibited by the previous addition of the Folin's reagent, should be completed immediately. In the event of positive results, indicating the presence of phenol or other foreign substances, the contaminated samples may be eliminated at once.

The control tests must be made under the same conditions of temperature and time of standing as the main test, for Folin's reagent produces a slow hydrolysis of the substrate, as is shown in Table VIII.

TABLE VIII

10 ml. of buffer-substrate + 0·5 ml. water + 4·5 ml. Folin's reagent. Sodium carbonate added after keeping the test at different temperatures for different times

Time of keeping	Temperature of keeping	Extinction coefficient k	Lovibond blue units
3 minutes	5°	0·093	0·4
24 hours	5°	0·240	1·1
24 hours	37°	0·410	2·2

PRACTICAL APPLICATION.—*Effect of time and temperature of pasteurisation.*—A few figures may be of interest to illustrate the results obtained (see Table IX).

TABLE IX

Temperature	Lovibond blue units			
63° C. for 30 minutes	2·0	2·2		
62° C. for 30 minutes	2·8	2·7		
Time				
10 minutes at 63° C.	4.5	—	—	—
20 minutes at 63° C.	3·3	2·7	3·4	2·7
30 minutes at 63° C.	2·1	2·0	2·2	2·1
40 minutes at 63° C.	1·9	2·0	—	1·8

These figures illustrate how sensitive the test is to variations possible in actual pasteurisation.

TABLE X

INFLUENCE OF STORAGE OF PASTEURISED MILKS

Milk Sample No.	Time of storage	Temperature °C.	k (S75)	Lovibond blue units
B	4 hours	Cold store	0·337	1·7
,,	7 days	,, ,,	0·307	1·6
,,	12 ,,	,, ,,	0·342	1·7
,,	15 ,,	,, ,,	0·334	1·7
D	0 hours	20	0·299	1·5
,,	24 ,,	20	0·314	1·6
,,	48 ,,	20	0·435	2·3
F	0 ,,	22	0·364	1·9
,,	24 ,,	22	0·378	2·0
,,	48 ,,	22	0·403	2·1
I	0 ,,	37	0·355	1·8
,,	20 ,,	37	0·547	3·8

Often it may be necessary to test a milk of unknown age. We have investigated whether prolonged storage affects the phosphatase-content of pasteurised milk. As shown in Table X, a milk sample kept at a low temperature will give the same result after a fortnight, whilst storage at a high temperature will give a higher reading according to the time and temperature. This increase in the colour may be due to the metabolic products of micro-organisms or to phosphatase of bacterial origin. Some knowledge of its history, therefore, is essential to an accurate judgment of a sample.

It has been found from the use of this test commercially over a period of 6 months that any interference by phosphatase of bacterial origin does not take place unless the milk samples have been kept under conditions which normally do not occur in this country.

Effect of admixture of raw milk.—According to our findings it is possible to detect an admixture of less than 0·25 per cent. of raw milk with legally pasteurised milk, as indicated in the following table:

TABLE XI

Raw milk added Per Cent.	Sample 1		Sample 2	
	k	Lovibond blue units	k	Lovibond blue units
0·00	0·328	1·7	0·332	1·7
0·10	0·423	2·3	0·498	2·8
0·25	0·498	3·1	0·667	4·0
0·50	0·871	6·1	0·945	6·6

CONCLUSIONS.—The phosphatase test of Kay and Graham for assessing the efficiency of pasteurisation of milk has been subjected to a detailed examination. The extent to which variations in the technique influence the result has been determined. As a result we find that, if satisfactory results are to be obtained with regularity, the published technique must be followed closely. Its practical application has been illustrated by reference to commercial samples.

We wish to thank the Directors of the United Dairies for their permission to publish that part of the work carried out in their laboratories, and Professor H. D. Kay for his interest in this investigation.

REFERENCES

1. Orla-Jensen, *Le Lait*, 1929, IX, 622.
2. H. D. Kay and W. R. Graham, *J. Dairy Res.*, 1935, 6, 191; Abst., ANALYST, 1936, 61, 44.
3. Circular 1533 of the Ministry of Health (April, 1936).

DISCUSSION

Professor KAY said that he had little to add to what Mr. Anderson had said. When he heard some little time ago that Mr. Anderson had given the method a searching trial and was finding it of real value he was very glad. He thought he ought to emphasise, what indeed Mr. Anderson had already implied, that it was

a delicate method of some precision and required competent laboratory handling to obtain results that could be relied upon.

One small point he felt he should mention—the quality of the filter-paper. It was advisable to use a No. 42 Whatman paper. No. 30 could be used, but, on the whole, he preferred No. 42. Another essential was that the buffer substrate solution should be kept saturated with chloroform. He also pointed out that, whereas the test was very satisfactory for pasteurised mixed milks (taking the standard for failure as anything above 2·3 Lovibond units), for the pasteurised milk of individual cows some caution was necessary. It was, of course, most unlikely that milk of individual cows would be separately pasteurised commercially, but he had come across one or two cases in which milk of individual cows, pasteurised at exactly 145° F. and for exactly 30 minutes in the laboratory, had given figures of 2·3 or 2·4. This had never occurred, however, with commercial samples and was probably an academic point only. The reason for it was that the phosphatase-content of raw milk in individual cows varied considerably from one cow to another. It varied in a fairly regular way with the period of lactation. In the first few days it might be very high, then it went down to a minimum and remained low for some weeks, then it rose very high again at the end of the lactation period. If one happened to get hold of milk from an individual cow at either end of the period of lactation, one might get figures of 2·3 units, even when it had been perfectly pasteurised.

Mr. R. C. FREDERICK said that, with a view to its employment for a particular purpose, he had made an extended investigation of the rapid form of the test, and had reached the conclusion that it was excellent. He found that so long as the conditions of storage were such that the milk remained palatable, so long at least was the test reliable. With regard to the minimum amount of unpasteurised milk that could be detected, he thought that this must depend upon the reaction for phosphatase given by the pasteurised milk with which it was admixed. It was most valuable that the authors should have made this critical survey of a test which would undoubtedly become standard.

Mr. R. W. SUTTON said that he was surprised to find in Table II that 0·25 per cent. of raw milk increased the blue units to about 6. Was this ordinary raw milk or was it a special one? Could the authors give any test for over-pasteurisation —at a temperature higher than that specified or for a longer time?

Dr. HERSCHDÖRFER replied that the raw milk added was the ordinary milk supplied to the United Dairies Laboratory and not a special one. Figures such as these were obtained on the addition of 0·5 per cent. of raw milk.

Mr. D. MOIR said that, in his experience, the average blue value of the controls when the phosphatase test was first started (early in the year) was running at about 1·5; since then it had increased in the summer months to about 1·7 to 1·8, and now it was usually as much as 2·0; 2·0 was very close to the limit of 2·3 suggested for genuinely pasteurised milks when submitted to the actual test. Had Mr. Anderson had any experience of milks which gave a control blue value of over 2·3? Mr. Moir said that he had recently examined a pasteurised milk having a control blue value of 2·6. To what was such a high blue value likely to be due?

Was the phosphatase completely destroyed if the milk was accurately pasteurised or was the final blue value in the test due to phenolic compounds in the milk and to residual phosphatase? Professor Kay had mentioned single cow milks being high in phosphatase and giving value slightly above 2·3. Presumably in such cases a little phosphatase was left. It had been shown that if milk were heated for a time only slightly less than 30 minutes or at a temperature only slightly lower than 145° F., a blue value considerably higher than 2·3 was obtained. Mr. Moir thought that, in view of these facts, the margin between the figures of 2·3 for correctly pasteurised milks and the control which might be as much as 2·0 was rather small.

Dr. HERSCHDÖRFER, replying, said that, of course, the phosphatase-content of raw milk was not constant, but, as a rule, 0·1 per cent. of raw milk was enough to give the test. If the raw milk was of very low phosphatase-content, however, it would require a higher percentage, but he had never had to add more than 0·2 per cent. As regards storage, when milks had been kept at a temperature not over 22° C., the kind of bacterial flora that developed seemed to have no influence on the readings.

Mr. A. PARKES referred to an experience in connection with a so-called pasteurised milk which gave a colour midway between those of raw and pasteurised milk; on investigation it was found that the milk had been held at pasteurising temperature for 2½ hours owing to the fact that the men had gone to lunch while it was being pasteurised. Could any explanation be given why the samples did not react to the test? The control gave the same colour.

Professor KAY remarked that it was most fortunate that the thermostability of milk phosphatase was such that it was completely or nearly completely destroyed when the legal minimum for pasteurisation time and temperature had been exactly fulfilled. Actually, if one pasteurised very accurately indeed at 145° C. for exactly 30 minutes, their findings showed that 1 part in 3000 or 4000 of the original phosphatase was left. In practice, the difficulty was to work at 145° C. exactly. Generally it was a little higher, so that the phosphatase in commercial practice was completely destroyed.

Regarding the short test—this had been put forward as a method of sorting out grossly under-heated milk and it worked very well for that. That was all that they had claimed. As to the amount of raw milk which could be detected, they had claimed originally 0·25 per cent.—they had been on the safe side, as workers in Denmark had recently submitted the test to careful scrutiny, and said that between 0·1 and 0·2 per cent. could be detected. There was no real difficulty with regard to over-pasteurisation of milk in commercial practice, because if milk was over-pasteurised, it affected the cream-line.

With reference to the question of getting above 2·3 in control tubes, that might be due to two or three things. They might have had some trouble with the filter-papers and that was a point which should be looked into. Another possible cause was if carbolic soap had been used by workers for washing their hands—this should not be used. A further possibility was that insufficient chloroform was added to the buffer substrate, and then one might get very slight hydrolysis of the substrate, giving free phenol in the control tubes.

The Detection of Arachis Oil in
Olive and Almond Oils

By NORMAN EVERS, B.Sc., F.I.C.

(Read at the Meeting, December 2, 1936)

THE British Pharmacopoeia method of detecting arachis oil in olive and almond oils depends on a sorting test and a confirmatory test. A positive result with the sorting test is not a certain indication of the presence of arachis oil; the separation of arachidic acid and the determination of its melting-point alone gives reliable evidence on this point.

OLIVE OIL.—The sorting test was originally proposed by Bellier,[1] and was subsequently modified by several workers, including Franz and Adler.[2] In 1912 I reported on an examination of this test,[3] which I found very satisfactory. Only one "neutralised" olive oil out of a number of oils tested gave a positive result.

Since that time the character of commercial olive oils has changed considerably, and a very large proportion of olive oils give a positive reaction. For the purposes of this paper 26 oils were selected as representative of the olive oils at present on the market. Of these oils, 13 were described as finest edible oils, 1 was a "refined" oil, 8 were of "pharmaceutical" quality, and 4 were "commercial" oils. The countries of origin, as far as they were known, included Spain, France, Italy, Greece, Syria, Tunis, and Turkey. Of these 26 oils, 16 gave a positive indication with the B.P. sorting test, but in only one of these was the presence of arachis oil confirmed.

The formation of precipitates by genuine olive oils in this test has been noted by Shelley[4] and by Droop Richmond and Powell,[5] among others. Powell proposed to remove the unsaponifiable matter and carry out the test on the fatty acids. This certainly diminishes the number of positive results, but makes the test considerably longer.

In the original test proposed by Bellier the oil was saponified with alcoholic potash, and acetic acid sufficient to neutralise the potash was added, followed by 50 ml. of 70 per cent. alcohol containing 1 per cent. of hydrochloric acid. The solution was then cooled to 17–19° C., and a precipitate showed the presence of 10 per cent. or more of arachis oil.

In the succeeding modifications the addition of hydrochloric acid to the 70 per cent. alcohol was omitted and only sufficient acetic acid was added to neutralise the potash used (or an amount slightly in excess of this). The resulting mixture gives a colour with methyl red corresponding to a pH of about 5·5 in aqueous solution, and the whole of the fatty acids are not set free. This is confirmed by the observation of Lüers[6] that olive oils giving a positive reaction contained an unusually high proportion of myristin, and that the precipitate

consisted of the acid potassium salt of myristic acid. He suggested adding an additional 3 drops of glacial acetic acid to prevent this.

With a view to the elimination of some of the work which is now necessary to prove the absence of arachis oil from olive oils I have re-investigated the qualitative test. In the first experiments I reverted to Bellier's original test, in which 1 per cent. of hydrochloric acid was added to the 70 per cent. alcohol. This at once reduced the clouding temperature of olive oils, but it was found that a reduction also occurred when arachis oil was present. Further experiments showed that the addition of acetic acid was not essential, and that the results depended entirely on the pH of the solution. If, for example, the saponified liquid was brought to the same pH by adding hydrochloric acid instead of acetic acid, identical results were obtained. Tests were therefore carried out, omitting the acetic acid and adding sufficient hydrochloric acid to set free the whole of the fatty acids, the reaction being definitely acid to methyl red.

In Fig. 1 are set out the results obtained on the series of 25 genuine olive oils and on the same oils with the addition of 5 per cent. of arachis oil by this modified test and by the B.P. method. In the B.P. test the solution is cooled to 15·5° C., and if no turbidity occurs within five minutes at this temperature arachis oil is regarded as absent. It will be seen that 15 (60 per cent.) of the oils gave a turbidity at or above 15·5°C. Further, if the critical temperature is raised above 15·5° C., 5 per cent. of arachis oil cannot be detected with certainty.

B.P. Test

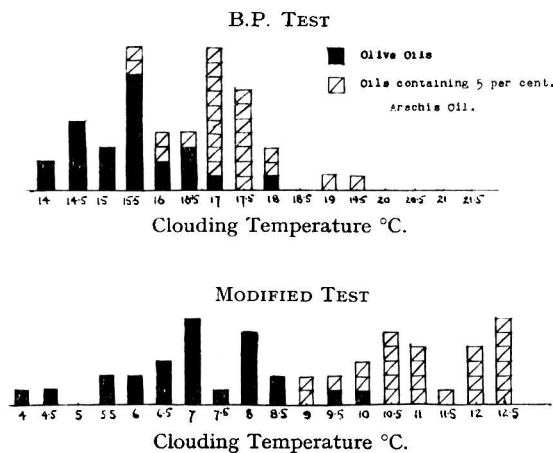Clouding Temperature °C.

Modified Test

Clouding Temperature °C.

Fig. 1.

In the modified test all but two of the olive oils gave no precipitate above 8·5° C., and all the oils containing 5 per cent. of arachis oil were turbid at 9° C. If, therefore, the solution is cooled to 9° C., 5 per cent. of arachis oil will be detected with certainty, and only a small proportion of pure olive oils will become turbid at this temperature.

The modified test is carried out in the following way:—One ml. of the oil is saponified with 5 ml. of 1·5 N-alcoholic potassium hydroxide solution by heating on a water-bath for five minutes, avoiding loss of alcohol; 50 ml. of 70 per cent.

alcohol are added, followed by 0·8 ml. of hydrochloric acid (sp.gr. 1·16).   After heating to dissolve any precipitate that may be formed the solution is cooled in water, stirring continuously with a thermometer, so that the temperature falls at the rate of about 1° C. per minute.   If a turbidity appears before the temperature reaches 9° C., the usual confirmatory test for arachis oil must be applied; if the liquid remains clear at this temperature, arachis oil may be regarded as absent.

It is essential that the stirring should be continuous, since local cooling will cause the premature formation of a turbidity.   For this reason the cooling water should not rise above the level of the liquid in the flask.

The turbidity temperature is best observed by looking through the liquid against a good light, and noting the temperature at which a definite precipitate first appears.   The point is quite sharp and the personal error should not be more than ±0·25° C.

Occasionally after acidification an oil gives a slight opalescence which is unaffected by warming.   This may be disregarded, as it does not affect the true turbidity temperature.

Table I shows the results obtained with oils other than olive, almond, or apricot-kernel oils.   The turbidity temperature of arachis oil itself is remarkably constant.   Thirteen samples of various origins and grades all gave turbidity temperatures of 39° to 40° C.   The test thus forms an excellent guide to the purity of arachis oil.

TABLE I

Oil	Clouding-point °C.
Arachis (13 samples)	39·0–40·0
Rape	22·5
Sesame	15
Cottonseed	13
Olive-kernel	10
Maize	7·5
Tea-seed A	9·5
Tea-seed B	2·5

In the absence of oils other than olive and arachis oils the turbidity temperature is a reliable quantitative measure of the arachis oil present.   Fig. 2 shows the results obtained by taking the two olive oils of the series shown in Fig. 1 which gave the highest and lowest turbidity temperatures, and adding varying proportions of 13 different arachis oils.   When the arachis oil is over 10 per cent. the turbidity temperature of the olive oil used does not exert any appreciable influence on the results.

ALMOND OIL.—The turbidity temperature of almond oil is lower than that of olive oil.   Consequently there is no likelihood of a genuine almond oil giving a positive result when arachis oil is absent either with the B.P. test or with the modified test as described above.   On the other hand, the clouding-point of almond oils is so low that the addition of 5 per cent. of arachis oil would escape detection. A series of almond and apricot-kernel oils has therefore been tested by the modified test, with and without the addition of 5 per cent. of arachis oil.   The results are given in Table II.

TABLE II

CLOUDING-POINTS OF ALMOND AND APRICOT-KERNEL OILS

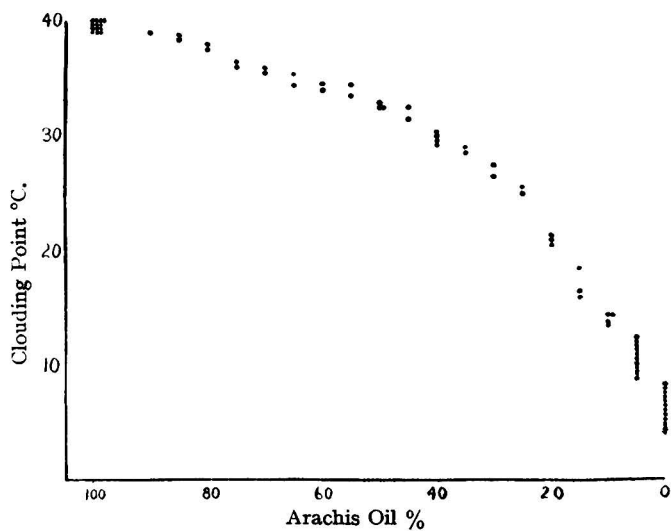| Oil			Clouding-point °C.	Clouding-point after addition of 5 per cent. of arachis oil °C.
Almond	..	..	A	1·0	5·5
		B	1·0	4·5
		C	1·5	4·5
		D	1·0	4·5
		E	0·0	4·5
		F	0·0	5·0
		G	−0·5	5·5
		H	−0·5	5·0
Apricot-kernel	..	J	−6·5	4·0
		K	−4·0	5·0
		L	−8·5	3·5
		M	−5·5	3·5

Fig. 2.

Oils A to E and J to M were authenticated samples and all English-pressed. Oil G was a French oil.

The addition of higher amounts of arachis oil to one of the genuine almond oils gave the following results:

Arachis oil added Per Cent.	Clouding-point °C.
0	1·0
5	4·5
7·5	9·5
10	15·0
20	20·5
30	26·5
50	32·5
75	36·5

It will be seen that when 10 or more per cent. of arachis oil is added, the results follow the curve in Fig. 2.

It seems necessary, therefore, when dealing with almond or apricot-kernel oils, to cool to 4° C. If a turbidity is formed, the confirmatory test must be carried out. Five per cent. of arachis oil will then be detected with certainty in the absence of other interfering oils.

I am indebted to Mr. E. R. Bolton, Messrs. Astor, Boisselier & Lawrence, Ltd., Leon Frenkel, Ltd., and Stafford Allen & Sons, Ltd., for kindly supplying samples of oil.

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DISCUSSION

Mr. E. M. HAWKINS said that he thought that the author's method might help in the detection of ground almonds. He had used the test on some oil extracted from Brazil nuts.

Mr. DAVIS mentioned that there were on the market olive oils, of North African origin, giving results below the B.P. titre. Had the author examined any of these oils?

Mr. C. E. SAGE said that the newer methods of preparing olive oil, by treating with chemical agents, oils with high acidities, yielded products which might be palatable and presentable at first sight, but they led to sophistication and admixture. Speaking generally, commercial olive oil had altered considerably in character in the last forty years. The Bellier test was not entirely reliable, and a complete Renard separation often yielded negative results, but some oils yielded crystals finally, which had to be considered arachidic acid, although they were derived from oils which were undoubtedly pure olive products. He had found that it was of the greatest importance to use pure alcohol, not industrial spirit, for the Bellier test.

Mr. E. R. BOLTON, in congratulating Mr. Evers on his communication, observed that until he had put before us the present test, analysts were in very many cases faced with conducting the full arachidic acid determination with its attendant melting-points, as being the only reliable way in which groundnut oil could be definitely detected and determined. The present test, however, clearly obviated the necessity of conducting this longer test so often.

Mr. Bolton also referred to the difficulties presented by "manipulated" oils and, as an example of such oils, cited the case of "green olive oils" which, having been extracted by carbon bisulphide, were very acid. The bulk of the free acids having been esterified with glycerin and the remaining acidity removed by

distillation, after subsequent refining and bleaching an oil was prepared bearing resemblance to the original olive oil and introducing great difficulties in the test. He enquired if Mr. Evers had applied his new method to the detection of arachis oil in such reconstituted oils.

Mr. J. R. Nicholls asked if this test had been applied to other substances, such as hardened fats. It seemed that with such fats the gradual lowering of the temperature might cause a considerable positive reaction.

Mr. Evers, replying, said that he had not examined oil from Brazil nuts; the work had been done entirely on olive and almond oils. The presence of ground pea-nuts in ground almonds, however, could be detected very easily by this method. In reply to the question about North African oils, he said that one oil which gave a precipitate at 9° C. was a Tunisian oil; another Tunisian oil came out below 9° C. It was very easy to recognise these oils because of the heavy nature of the precipitate. He had not applied the test to hardened fats, but thought that it would not be satisfactory. He did not know whether any of the oils tested were "manipulated" oils, but a few of them might have been. The rate of cooling was a matter of great importance. He allowed the temperature to fall at the rate of 1° C. per minute, starting with warm water and then adding ice.

The Determination of Cyanide in Aqueous Extracts of Road Tars

By W. G. MOFFITT, Ph.D., A.I.C., and E. H. WILLIAMS, B.Sc., A.I.C.

(Read at the Meeting, December 2, 1936)

INTRODUCTION.—It has been shown by Southgate, Pentelow and Bassindale[1] that cyanide, in concentration above 0·01 part of CN per 100,000, is toxic to fish. Rain washings from recently tarred roads may gain access to fishing rivers, and it is important that they should not contain cyanide to this extent.

In the examination of a road tar the determination of cyanide is made on an aqueous extract, prepared under standard conditions (*vide infra*), and the concentration of cyanide in this extract is regarded as indicating the maximum that might be expected to occur in the rain washings from a recently tarred road.

The only method of determining cyanide found to be sufficiently sensitive is that described by Childs and Ball[2] (see also Weehuizen),[3] which is based upon the oxidation of phenolphthalin to phenolphthalein by cyanide in the presence of a cupric salt. This method is affected by the presence of phenolic compounds, and the following table shows how the presence of tar acids, prepared from a fraction of coal-tar creosote (205–230° C.), affects the test.

Childs and Ball (*loc. cit.*) state that by distilling under reduced pressure the quantity of phenols in the distillate will be reduced. When acidified solutions of potassium cyanide, containing appreciable quantities of tar acids, were distilled

under reduced pressure, it was not found possible to obtain good yields of cyanide free from tar acids, and other methods were sought for preparing a pure solution of cyanide from the aqueous extract of a road tar.

TABLE I

THE EFFECT OF TAR ACIDS ON THE PHENOLPHTHALIN TEST FOR CYANIDE

Tar acids added (parts per 100,000)	0·0	0·05	0·10	0·50	1·00	2·00
Apparent cyanide (parts of CN per 100,000)	0·016	0·014	0·012	0·008	0·008	0·006

Attempts were first directed to removing the tar acids from the test solutions by extraction with solvents such as chloroform, carbon tetrachloride, benzene and petroleum spirit.   When pure benzene or carbon tetrachloride is shaken with water the aqueous layer gives a positive reaction with the phenolphthalin reagent in the absence of added cyanide.   Petroleum spirit has the opposite effect of slightly inhibiting the reaction.   Chloroform is without effect on the phenolphthalin test, but it extracts a little of the hydrogen cyanide from the aqueous layer, particularly in the presence of a strong acid.   When the neutral distillates from artificial mixtures of potassium cyanide and tar acids were extracted with chloroform the yields of cyanide obtained were consistently about 85 per cent. of the theoretical. When, however, this method was applied to neutral distillates from aqueous extracts of road tars, containing added quantities of cyanide, the cyanide determined by the phenolphthalin test varied from nil to 40 per cent. of the total (see Table II, col. 3).

TABLE II

Parts per 100,000

		Yield of cyanide	
1 No. of extract	2 Cyanide added to extract	3 (a) after extraction of distillate with CHCl₃	4 (b) after oxidation of distillate and further distillation
1	Nil	—	0·003
	0·008	0·003	0·003
2	0·003	Nil	0·0025
	0·008	0·001	0·005
3	0·0065	0·0015	0·0015

Since the final test is essentially an oxidation reaction, it seemed probable that, if the interfering substances in the aqueous extract could be preferentially oxidised before distilling off the cyanide, the resulting distillate would be free from interfering substances.   Good results were obtained when artificial mixtures of cyanide and tar acids were oxidised with a slight excess of permanganate in the presence of phosphoric acid.   When aqueous extracts of road tar, containing added quantities of cyanide, were oxidised in this way the yields of cyanide were often in excess of the theoretical, presumably owing to the oxidation of thiocyanate to cyanide.   An alternative method was tried whereby the aqueous extract was first acidified with tartaric acid and then distilled.   The distillate, which contained no thiocyanate, was oxidised at 20° C. with a slight excess of permanganate.   A

reducing agent, *e.g.* manganous chloride, was added before finally distilling off the hydrogen cyanide, in order to prevent the oxidation of cyanide by the excess permanganate at the boiling temperature. The results obtained by this method are given in Table II, col. 4.

These results (Table II, col. 4) suggest that there is an appreciable loss of cyanide on distilling the unoxidised tar extract. In order that the oxidation might be carried out on the tar extract before distillation, it was necessary to use an oxidising agent which would not oxidise the thiocyanate to cyanide.

Silver oxide proved to be a suitable oxidising agent when precipitated *in situ.* There is a loss of cyanide when silver oxide is precipitated from the nitrate, and better results are obtained by precipitating from silver acetate. Silver oxide will oxidise cyanide on boiling, and it is necessary to destroy excess of oxide before distilling, by adding a solution of stannous chloride. The distillate contains appreciable quantities of unoxidised tar acids and naphthalene, which must be removed by extraction with chloroform before applying the test.

Silver oxide has a slight oxidising effect on thiocyanate, which will only interfere, however, when the concentration of thiocyanate is more than a hundred times that of the cyanide. A modification of the method is described (*vide infra*) which gives reliable figures for cyanide in the presence of 1 part per 100,000 of thiocyanate. It is based on the precipitation of the thiocyanate as the silver salt in the presence of a little acid, which retards the reduction of the precipitated silver thiocyanate by the aqueous extract. The precipitated silver thiocyanate is not oxidised by silver oxide.

METHOD.—The aqueous extract of the road tar is prepared by shaking a known weight of the tar, approximately 100 g., spread over the bottom of a Winchester quart bottle, with 15 times its weight of cold, fully aerated, distilled water for 16 hours on a mechanical shaker at a temperature between 15–20° C. The extract is then filtered through a single 12-inch, fluted filter-paper.

The solutions required in the preparation of the solution for the test are as follows:—(A) A saturated solution of silver acetate, (B) a 1 per cent. solution of caustic soda, and (C) a 5 per cent. aqueous solution of stannous chloride containing 2·5 per cent. by vol. of conc. hydrochloric acid.

The distillation is carried out in a glass-stoppered distilling flask with a side-arm fitting by means of a ground-glass joint into a Liebig condenser. In place of the ground-glass joints rubber bungs covered with metal foil (tin or aluminium) may be used.

The filtered extract (250 ml.) is run into the distillation flask, and 50 ml. of saturated silver acetate solution are added. The contents of the flask are gently shaken while 12 ml. of 1 per cent. caustic soda solution are run in. The flask is then set aside for 15 minutes, after which 20 ml. of the stannous chloride solution are added and the distillation is begun. The distillate, about 75 ml., is made up to 100 ml. with distilled water, transferred to a dry separating funnel, and extracted three times with 10 ml. quantities of chloroform. The aqueous layer is transferred to a dry flask and, after standing for about 10 minutes, an aliquot portion, usually 50 ml., is taken for the test.

The following modification of this method should be used when the quantity of

thiocyanate present is such as to give a distinct reddish colour on adding a drop of ferric chloride solution to 100 ml. of the extract slightly acidified with acetic acid. To 250 ml. of the filtered extract, in the distillation flask, are added 1 ml. of N acetic acid and 5 ml. of saturated silver acetate solution, and the flask is set aside in the dark for 30 minutes. A further quantity of 45 ml. of silver acetate solution is then added, followed by 12 ml. of 1 per cent. caustic soda solution and, after 15 minutes, 20 ml. of stannous chloride are added, and the solution is distilled and extracted as described above.

The reagents for the phenolphthalin test for cyanide are those given by Childs and Ball (*loc. cit.*, p. 298),* but a greater quantity of distillate is taken for the test.

The aliquot portion taken is diluted to 100 ml., and 1 ml. of the phenolphthalin reagent is added, followed by 2 ml. of 1 per cent. caustic soda solution. A red colour develops and is compared with that produced in solutions containing known quantities (0 to 0·015 parts of CN per 100,000) of cyanide treated in an identical manner.

The results (as parts per 100,000) obtained by this method with nine samples of road tars are given in Table III. The determinations were carried out on the fresh extracts, and, as the quantities of cyanide found were small, second determinations were made after addition of potassium cyanide, equivalent to 0·01 part of CN per 100,000, to the extracts. The figures in column 5 are the results obtained when the oxidation with silver oxide was omitted.

TABLE III

1 Tar examined	2 Cyanide added to aqueous extract	3 Tar acids found in extract	4 Cyanide found after oxidation, distillation and extraction	5 Cyanide found after distil- lation and extraction alone
A	—	0·4	Nil	—
	0·010		0·0075	—
B	—	1·8	0·0003	—
	0·010		0·009	—
C	—	2·4	0·001	—
	0·010		0·009	—
D	—	2·4	Nil	—
	0·010		0·008	0·0035
E	—	4·4	0·0005	—
	0·010		0·0095	0·004
F	—	5·0	0·0015	—
	0·010		0·0085	—
G	—	9·0	0·001	—
	0·010		0·009	—
H	—	28·0	0·001	—
	0·010		0·0095	0·001
I	—	40·6	0·001	—
	0·010		0·009	—

* To prepare the reagent for the test, 10 ml. of a 1 per cent. solution of phenolphthalin containing 0·2 per cent. of sodium hydroxide are diluted to 50 ml. with a 2·5 per cent. solution of glycerol. To this are added 50 ml. of a 0·3 per cent. solution of copper acetate, and the whole is well mixed, filtered (if cloudy), and kept in a stoppered glass bottle.

The results obtained by the modified method, for extracts containing appreciable quantities of thiocyanate, are given in Table IV. As none of the extracts examined contained sufficient thiocyanate to test the method, potassium thiocyanate, equivalent to 1·0 part of CNS per 100,000, was added, and the test was applied to this mixture, with and without additional cyanide. The last two experiments, with solutions of thiocyanate in water, show that there is very little, if any, oxidation of the thiocyanate by this method.

TABLE IV

YIELDS OF CYANIDE OBTAINED IN THE PRESENCE OF THIOCYANATE

(Parts per 100,000)

Solution used	Thiocyanate added	Cyanide added	Cyanide found
Extract A	—	—	Nil
,,	1·00	—	0·0015
,,	1·00	0·01	0·011
Extract I	—	—	0·001
,,	1·00	—	0·0015
,,	1·00	0·01	0·008
Water	1·00	—	0·0007
,,	1·00	0·01	0·009

The method has been applied to artificial mixtures of cyanide with various phenols and the results are shown in Table V.

TABLE V

THE EFFECT OF ADDING PHENOLIC SUBSTANCES TO SOLUTIONS OF CYANIDE

1 Cyanide present (parts per 100,000)	2 Substance added	3 Quantity added (parts per 100,000)	4 Yield of cyanide after distillation and extraction Per Cent.	5 Yield of cyanide after oxidation, distillation and extraction Per Cent.
0·01	Purified tar acids ⎰	100	—	80
0·01	from creosote ⎱	6	85	—
0·01	Phenol	100	—	80
0·01	o-Cresol	40	50	70
0·01	p-Cresol	40	60	80
0·01	1.2.3-Xylenol	40	65	80
0·01	1.3.5-Xylenol	40	70	80
0·01	Pyrogallol	40	Nil	75
0·01	Hydroquinone	5	Nil	75
0·01	Catechol	2	Nil	85
0·01	Catechol	10	—	60
0·01	Resorcinol	40	70	80
0·01	α-Naphthol	16	50	80
0·01	β-Naphthol	1	40	70
0·01	β-Naphthol	10	—	50

It will be seen that amounts up to 40 parts of the more common phenols per 100,000 are without appreciable effect on the method involving preliminary oxidation. The results in column 4 show the effects of these substances when

oxidation by silver oxide is omitted. The di- and tri-hydroxy phenols have the greatest effect. Catechol and β-naphthol, which when present in considerable quantity have a marked effect on the method, have been found in coal tars, but normally represent only a very small fraction of the total phenols (*cf.* Edwards[4] and Currey[5]).

SUMMARY.—Conditions are given whereby cyanide in aqueous extracts of road tars, in quantities of 0·01 part per 100,000, can be determined with reasonable accuracy. The tar acids are removed by oxidation with silver oxide, followed by distillation and subsequent extraction of the distillate with chloroform.

We wish to thank Dr. J. J. Fox, the Government Chemist, for permission to publish this work, and Mr. W. G. Adam, of the Gas, Light and Coke Company, for kindly supplying samples of road tars.

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DISCUSSION

The PRESIDENT said that this interesting paper was of importance in relation to the poisoning of fish. He asked whether it was a fact that this poisoning usually occurred when the tar was newly laid and again when the surface was breaking up and the road required re-tarring.

Mr. WILLIAMS replied that this was so.

Dr. H. E. Cox remarked that the authors had made a notable contribution to a difficult problem. There was no doubt that the phenolphthalin test was the most sensitive one available for demonstrating the presence of cyanide, but it was not specific. The authors had been dealing with such extremely minute quantities as 1 in 200 millions; in streams where fish had died he thought that cyanide could be inferred from the red colour in the phenolphthalin test without other confirmatory tests.

Dr. MOFFITT replied that essentially the method had been worked out on tar. River water would first be distilled, and if one obtained a pink colour in the distillate he thought that one might attribute it to cyanide. He did not think that anything else in the distillate would give the reaction.

Mr. C. E. SAGE asked whether the authors had applied their method to the detection of traces of hydrocyanic acid in effluents, particularly those where land had been dressed with ammonium sulphate, or where there was drainage from piles of vegetable matter containing a cyanogenetic glucoside.

Mr. WILLIAMS replied that there had been cases of the poisoning of fish due to drainage from tarred roads and that the purpose of this test was to test the coal-tar itself before it was applied to roads draining into streams. The work was done in order to define a "non-toxic" tar conforming with a standard specification. It was not done from the point of view of investigating fish poisoning.

Mr. A. L. BACHARACH asked whether the difference between the recovery of hydrocyanic acid alone, when it was 100 per cent., and in the presence of tar acids, when it was noticeably less, might not be due to some hydrolysis to formic acid.

Dr. MOFFITT replied that there was a slight loss, amounting to 5 to 10 per cent. of the cyanides present, but they must remember that the acid was very weak. Undoubtedly, with strong acid, there was hydrolysis, but here the distillation was carried out in the presence of buffered acetic acid.

The Determination of Molybdenum in Cast Iron

By E. TAYLOR-AUSTIN, A.I.C.

INTRODUCTION.—The problem of suitable methods for the determination of molybdenum has been greatly accentuated in recent years by the increasing use of this element as an alloy addition to cast iron and steel. At the present time two methods are in general use for the determination of molybdenum in cast iron— the lead molybdate method and the sulphide method. Recently, following on the work of Knowles[1] and others, α-benzoinmonoxime has been introduced for the general determination of this element. The first published paper on the application of this reagent to cast iron appears to be that of Thompson and Stott.[2]

This work is a study of all three methods as applied to cast iron, and the conclusion to be drawn is that, although with certain modifications in procedure equally reliable results can be obtained by all the methods, the α-benzoinmonoxime method is the most satisfactory. Incidentally, the time taken for a determination by the latter method is $1\frac{1}{2}$ to 2 hours, as compared with 4 to 5 hours by either of the other methods.

THE LEAD MOLYBDATE METHOD.[3]—Long experience with this method, extending over a period of several years, has shown that when several determinations are made on the same sample of cast iron, the results do not always fall within the recognised limits of ± 0.02 per cent. of molybdenum, and, further, that the method occasionally yields low results. Also, the lead molybdate precipitate sometimes appears immediately on addition of the ammonium acetate solution and is granular, filtering well and scarcely adhering to the precipitation vessel, whilst on other occasions the precipitate does not appear until the solution has been boiled for several minutes after the addition of ammonium acetate solution, and is then in a finely divided state, tending to "creep" over the sides of the filter-funnel and adhering tenaciously to the vessel. This is not due to variations in the amount of molybdenum present, since duplicates of the same sample may exhibit these differences.

In this section an attempt is made to discover the cause of these irregularities and to prevent their occurrence by standardising or otherwise improving the conditions of precipitation. Since it is necessary to remove iron before the precipitation of molybdenum by this method, in the early stages of the work the

conditions of precipitation from pure solutions were studied, a standard solution of pure molybdic oxide (AnalaR) in dilute sodium hydroxide being used.

THE EFFECTS OF AMMONIUM ACETATE CONCENTRATION.—The conditions of precipitation were made as nearly constant as possible in the following manner:— The equivalent of 0·68 per cent. of molybdenum (for a 1-gram sample) was added to a volume of 300 ml. of distilled water and the solution was made just acid to litmus with conc. hydrochloric acid and heated to boiling-point. Next 20 g. of ammonium chloride were added, and the boiling was continued for five minutes, after which 10 ml. of a 4 per cent. solution of lead acetate* were added, the resulting precipitate was just dissolved in hydrochloric acid, and 0·20 ml. in excess was added from a burette. The solution was again boiled and amounts of ammonium acetate solution,† varying from 5 to 80 ml., were added. After being boiled for 1 minute, or longer (if necessary to produce a precipitate), the solution was filtered, and the precipitate was washed with hot water, ignited and weighed. The results are shown in Table I.

TABLE I

Ammonium acetate solution added ml.	Weight of PbMoO$_4$ obtained (calculated PbMoO$_4$ from Mo taken 0·0260 g.) g.	Time taken for precipitate to appear after the addition of ammonium acetate Seconds
5	0·0260	2
10	0·0260	4
20	0·0258	6
30	0·0255	15
40	0·0240	25
50	0·0238	30
60	0·0230	55
70	0·0235	65
80	0·0230	75

It will be seen that under these conditions 5 ml. of ammonium acetate solution, as compared with 20 ml. recommended in the original method,[3] are sufficient to precipitate all the molybdenum added. As the quantity increases up to 80 ml. the results drop slightly, and it was also observed that the precipitates became less granular, although they showed no tendency to pass through the filters. Further, with 5 ml. of ammonium acetate solution the precipitates appeared almost immediately, but, as the amount of this reagent increased, the "time lag" of precipitation also increased, up to 75 seconds with 80 ml. of ammonium acetate solution.

THE EFFECTS OF HYDROCHLORIC ACID CONCENTRATION.—It was found that if the excess of hydrochloric acid, referred to above, was increased even by 0·10 ml., the amount of ammonium acetate solution required to produce a precipitate of lead molybdate within a given time increased very considerably, and the precipitates were less granular and required a much longer time for making their appearance. This effect is shown in Table II, and it will be seen that with an excess

* Made by dissolving 40 g. of lead acetate in distilled water, just clearing the white precipitate formed with 30 per cent. acetic acid and diluting to 1 litre.

† Made by adding ammonium hydroxide (sp.gr. 0·880) to 30 per cent. acetic acid until neutral to litmus.

of 0·6 ml. of hydrochloric acid a precipitate could not be obtained in less than 60 seconds. Furthermore, with an excess of more than 0·3 ml. of acid the precipitates showed a pronounced tendency to pass through the filters. Thus, the best conditions appear to be established when 0·2 ml. excess of acid and 5 ml. of ammonium acetate solution are employed, the results obtained being consistently accurate within the limits of analytical tolerance.

TABLE II

Hydrochloric acid added in excess ml.	Ammonium acetate required to yield a precipitate in less than 60 seconds ml.	Time for precipitation after addition of acetate Seconds
0·2	5	2
0·3	15	20
0·4	25	35
0·5	40	50
0·6	70	70

The conditions of precipitation are far evidently more exacting than the earlier literature on this method would suggest.

PRECIPITATION FROM ACETIC ACID SOLUTIONS.—In order to avoid, if possible, these rigid conditions and thus to simplify the method, a modification was introduced at this stage. It consisted in precipitating the molybdenum from solutions containing acetic acid, which is only slightly dissociated in solution, and thus avoiding the use of both hydrochloric acid and ammonium acetate. The equivalent of 0·68 per cent. of molybdenum was again added to 300 ml. of distilled water, but the addition of ammonium chloride was omitted. The solution was then made just acid to litmus with acetic acid (30 per cent.), and amounts of excess acid varying

TABLE III

Excess 30 per cent. acetic acid added ml.	Lead molybdate obtained (calculated PbMoO$_4$ from Mo taken 0·0260 g.) g.	Time taken for precipitate to appear after addition of lead acetate solution
Nil	0·0272	
10	0·0264	
20	0·0260	
30	0·0262	Immediate
40	0·0258	precipitation
50	0·0262	in every
60	0·0262	instance
70	0·0262	
80	0·0260	

from nil to 80 ml. were added. It was then heated to boiling, and the molybdenum was precipitated by the addition of 20 ml. of lead acetate solution (4 per cent.). After being boiled for 1 minute the solution was filtered, and the precipitate was washed with hot water, ignited, and weighed as before. The results obtained are shown in Table III, and are seen to be very consistent, the greatest difference

between 8 determinations being less than 1 mg., with the exception of the first case, *i.e.* where little or no excess of acetic acid was present; under these conditions it appears probable that a little lead hydroxide was precipitated. Thus the whole of the molybdenum added is precipitated over a wide range of acetic acid concentrations.

When, however, the procedure described above was repeated with the addition of 20 g. of ammonium chloride the precipitates became less granular and showed a marked tendency to pass through the filters, more particularly on washing. In order to avoid this difficulty a little paper pulp was added prior to filtration. This reduced very considerably the tendency to "run through," although it did not prevent it entirely. It was found, however, that if a hot 2 per cent. solution of acetic acid was substituted for hot water as the washing medium, this difficulty was entirely removed, and the results became even more consistent than those obtained by the hydrochloric acid and ammonium acetate method with rigid control of conditions.

THE EFFECTS OF PHOSPHATE ION ON THE PRECIPITATION OF MOLYBDENUM IN ACETIC ACID SOLUTION.—The modified method described was then investigated more fully, particularly with reference to interference by the phosphate ion and the prevention of such interference by the addition of ammonium chloride. The same standard molybdenum solution as before was used, and to it was added a standard solution of pure (AnalaR) sodium phosphate. The precipitations were carried out in a volume of 300 ml. of distilled water containing 20 ml. excess of 30 per cent. acetic acid. No ammonium chloride was added at first. The precipitates were washed with hot 2 per cent. acetic acid solution, ten washes of which were sufficient to remove all the lead salts; the filtrates were tested from time to time with potassium dichromate solution. Since the true weight of lead

TABLE IV

Phosphorus added Per Cent.	Lead molybdate obtained (calculated weight of PbMoO$_4$ 0·0260 g.) g.	Increase in weight due to phosphorus g.	Increase in weight due to 0·26 per cent. increase in phosphorus g.
0·26	0·0410	0·0150	—
0·52	0·0692	0·0432	0·0282
0·78	0·0970	0·0710	0·0278
1·04	0·1244	0·0984	0·0274
1·30	0·1525	0·1265	0·0281
1·56	0·1776	0·1516	0·0251

molybdate, for any given amount of the standard solution of molybdenum added, was known accurately from previous experiments, the amount of interference due to phosphorus was calculated by difference.

In the first series examined the molybdenum-content of the solution was kept constant at 0·68 per cent., and the phosphorus-content varied from 0·26 per cent. to 1·56 per cent., and the results are shown in Table IV. It will be seen that the weight of the precipitate increases with the amount of phosphorus present, and furthermore this increase is constant for every 0·26 per cent. added. The direct relationship observed between the phosphorus-content and the increase in weight

of the precipitate, under the above-mentioned conditions, is so striking that it is unlikely that any lead phosphomolybdate is formed, so that one theory of phosphorus interference (*viz.* that it is due to the formation of phosphomolybdate) must be untenable.

In the second series examined, the amount of phosphorus present was kept constant at 1·0 per cent. and the molybdenum content varied from 0·13 per cent. to 2·66 per cent.; the results are given in Table V.

TABLE V

Molybdenum added Per Cent.	Calculated equivalent weight of PbMoO$_4$ g.	Weight of precipitate obtained g.	Increase in weight due to phosphorus g.
0·13	0·0051	0·1014	0·0963
0·27	0·0102	0·1067	0·0965
0·40	0·0152	0·1122	0·0970
0·53	0·0203	0·1177	0·0974
0·67	0·0254	0·1222	0·0968
1·33	0·0510	0·1480	0·0970
2·00	0·0764	0·2205	0·1441
2·66	0·1020	0·2602	0·1582

The weight of precipitate obtained, in excess of the calculated weight of lead molybdate, in this case, is seen to be constant up to a molybdenum-content of 1·33 per cent., but above this amount the weight of precipitate due to phosphorus increases with the amount of molybdenum present.

THE EFFECTS OF AMMONIUM CHLORIDE.—Again, the equivalent of 0·68 per cent. of molybdenum was added to a volume of 300 ml. of distilled water containing 1·0 per cent. of phosphorus, added as phosphate, and followed by amounts of ammonium chloride varying from 5 g. to 30 g.   The precipitations were carried out exactly as before, and the results are shown in Table VI.   It will be seen that with

TABLE VI

Ammonium chloride added g.	Weight of precipitate obtained (calculated PbMoO$_4$ from Mo taken, 0·0260 g.)		
	P = 0·50 per cent. g.	P = 1·0 per cent. g.	P = 1·5 per cent. g.
5	0·0804	0·1572	—
10	0·0800	0·1588	—
15	0·0644	0·1470	—
20	0·0362	0·1170	0·1583
25	0·0259	0·0260	0·1054
30	0·0262	0·0262	0·0447
35	0·0260	0·0260	0·0258
40	0·0258	0·0262	0·0260
45	0·0264	0·0262	0·0259
50	0·0262	0·0258	0·0262

these amounts of molybdenum and phosphorus the addition of ammonium chloride has little or no effect until more than 15 g. have been added; 20 g. of the reagent reduce the interference due to phosphorus by about 25 per cent., whilst 25 or 30 g.

reduce it entirely and give a figure consistent with that obtained from phosphorus-free solutions.

These experiments were repeated with the same amount of molybdenum but with 0·5 per cent. and 1·5 per cent. phosphorus. Ammonium chloride in amounts up to 50 g. was subsequently added, and the results are shown in Table VI.

With a phosphorus concentration of 0·5 per cent. it will be observed that the theoretical figure is obtained again with 25 g. of ammonium chloride, but with 1·5 per cent. of phosphorus 35 g. are necessary.

Finally, since with more than 1·3 per cent. of molybdenum the extent of the phosphorus contamination increases with the addition of both molybdenum and phosphorus, another series was examined containing 4·0 per cent. of molybdenum and 1·5 per cent. of phosphorus. For these quantities 35 g. of ammonium chloride proved sufficient, although, as will be seen from the lower portion of Table VI, an excess of this reagent in no way impairs the results.

The modified method, which is described in detail below, was then applied to several samples of various types of alloy cast iron, and the results are shown in the Appendix.

THE MODIFIED LEAD MOLYBDATE METHOD.—Dissolve 2 g. of drillings in 35 ml. of conc. hydrochloric acid. When the action subsides, oxidise the solution with a few ml. of conc. nitric acid and boil off nitrous fumes; filter off the silica, graphite, etc., on a paper-pad filter and wash well with hot water. Transfer the residue and pad to a 750-ml. conical flask containing 150 ml. of 2 N sodium hydroxide solution and heat to boiling. Meanwhile, to the filtrate add dilute sodium hydroxide solution until the colour becomes a deep red-brown, but the liquid is still entirely free from precipitate. Warm this solution to about 60° C. and then run it slowly from a separating funnel into the flask containing the boiling sodium hydroxide solution and the original residue. The flask should be shaken continually throughout this procedure. When the whole of the solution has been introduced, shake the flask well, and transfer the contents to a 500-ml. measuring flask, dilute with hot water to the graduation-mark, and add a further 2 ml. of water to allow for the volume of the precipitated matter. Pour the solution back into the original flask, after shaking, and note its temperature. After allowing the precipitate to settle somewhat, filter off 250 ml. (equivalent to 1 g. of iron) through a double fluted filter-paper, and adjust the volume of this solution to the graduation-mark at the temperature previously recorded, i.e. at the temperature at which the solution was diluted to 500 ml. Transfer the solution to a 600-ml. beaker, and add 30 per cent. acetic acid until the liquid is just acid to litmus paper, followed by an excess of 20 ml. of this acid. Then add 25 to 30 g. of ammonium chloride, according to the phosphorus-content of the sample, heat the solution to boiling, add 20 ml. of 4 per cent. lead acetate solution (previously described), and continue boiling for 1 minute. Filter the liquid through a tight paper-pad filter and wash the precipitate 10 times with hot 2 per cent. acetic acid solution. Ignite the washed precipitate at a temperature not exceeding 600° C., and weigh as lead molybdate (which contains 26·16 per cent. of Mo).

Several of the precipitates obtained by this method from samples of cast iron were dissolved in 10 ml. of conc. hydrochloric acid, and the solutions were boiled,

diluted and filtered. The precipitates were washed well with hot water, the filtrates were first made alkaline with sodium hydroxide and then acid with 30 per cent. acetic acid, exactly as before, and the molybdenum was precipitated with lead acetate solution as previously described. The weights of precipitates so obtained, with or without the addition of more ammonium chloride, were consistently the same as those of the original precipitates. Thus, provided that 25 g. to 30 g. of ammonium chloride are used, a re-precipitation is unnecessary.

THE SULPHIDE METHOD.[4]—This method is, of course, unaffected by phosphorus, but when molybdenum is precipitated in the presence of large amounts of iron (as in the analysis of cast iron) the precipitate obtained is invariably contaminated with iron. Purification may be carried out by any of the following methods:—

(i) Fuse the impure molybdic oxide with sodium carbonate, extract with hot water, filter off the ferric hydroxide so obtained, and ignite and weigh.

(ii) Boil the impure oxide with 20 ml. of 20 per cent. sodium hydroxide solution for 3 to 5 minutes, dilute to about 150 ml., and again boil for 5 minutes. Filter, wash the precipitate well with hot water, ignite and weigh the ferric oxide so obtained.

(iii) Acidify the filtrates obtained, after removal of iron, by either of the above-mentioned methods, with 30 per cent. acetic acid, add 20 ml. of acid in excess, and precipitate the molybdenum with lead acetate exactly as described in the modified lead molybdate method.

The weight of the iron residue obtained by either method (i) or (ii) is deducted from the weight of the impure oxide to obtain the true molybdenum figure. The method of treating the precipitate with 20 per cent. sodium hydroxide solution is much quicker than the fusion method and quite as satisfactory. Further, it avoids the use of platinum apparatus. It was found, however, when using this method, that there was a great tendency for some iron to pass through the filter. This may be avoided by diluting the solution well and boiling for about five minutes to coagulate the ferric hydroxide precipitate, as stated above.

The method of calculating the molybdenum percentage direct from the corrected weight of molybdic oxide obtained is open to serious objection. Owing to the high molybdenum content of the oxide (66·67 per cent.) any small errors in weighing or estimating the iron impurity to be deducted cause a large error in the final result. This can be overcome by using samples of 3 g. or more, but the precipitates of iron impurity obtained prove unwieldy by this method and it is far better to precipitate the molybdenum as molybdate, as stated in method (iii) (*supra*).

It is a rather significant fact that, in 14 of 16 determinations carried out by the sulphide method, which is generally reputed to yield low results, the actual figures obtained were slightly higher than those given by the modified lead molybdate method and the benzoin-oxime method for the same samples. The results for one particular case are shown in the Appendix.

The procedure described by Lundell, Hoffman and Bright,[5] in which the molybdenum is first oxidised with ammonium persulphate and then removed as sulphide in the presence of tartaric acid, was carried out a number of times on samples of 2, 3, and 5 g. Great difficulty was experienced in filtering off the

molybdenum sulphide so obtained, and in every instance the precipitates fused on ignition, despite copious washing. In view of the close agreement obtained between results by the more simple sulphide method[4] and those by the other two methods cited, further work on the method of Lundell and his co-workers was abandoned.

THE α-BENZOINMONOXIME METHOD.—In mineral acid solution α-benzoin-monoxime yields precipitates with the following: molybdenum, tungsten, chromium (sexivalent), vanadium (quinquevalent), tantalum and niobium. Silicon interferes somewhat, and should be removed before estimating any of the above-mentioned elements.

The following metals are not precipitated: silver, lead, mercury, bismuth, copper, cadmium, arsenic, antimony, tin, aluminium, iron, titanium, zirconium, chromium (tervalent), vanadium (quadrivalent), cerium, uranium, nickel, cobalt, manganese, zinc, selenium, tellurium, rhenium, ruthenium, rhodium, osmium, indium and platinum.[6]

The α-benzoinmonoxime method possesses several marked advantages over the two methods already studied:

(i) Samples of 3 g. or more may readily be dealt with; the sodium hydroxide separation incorporated in the lead molybdate method is impracticable if more than 2 g. of iron are present.

(ii) It provides a complete separation, in one operation, of molybdenum from all elements commonly found in cast iron, with the exception of silicon, which is, however, readily removed prior to precipitation. Thus nickel, titanium, phosphorus, manganese and iron do not yield precipitates under any conditions; chromium and vanadium are not precipitated in their reduced states, nor copper in acid solutions.

(iii) In the lead molybdate method a sodium hydroxide separation is necessary to remove iron, whilst if vanadium is present, special treatment, involving the use of manganese chloride, must be adopted; in the sulphide method the molybdenum is still associated with the other elements of the hydrogen sulphide group, e.g. copper, arsenic, etc. The use of α-benzoinmonoxime thus avoids many tedious separations, especially when dealing with complex alloy cast irons.

CONDITIONS OF PRECIPITATION.—*Permissible acidity.*—In order to prevent the interference of certain elements, the solution from which the molybdenum is precipitated must be distinctly acid. In the method given in detail later, a sulphuric acid solution is employed, but, should the presence of this acid prove objectionable, equally good results may be obtained with hydrochloric or nitric acid solutions of the same concentration by volume. Tartaric acid should be absent, as the precipitations are incomplete in its presence. Hydrofluoric acid is also to be avoided. In the method published by Thompson and Stott[2] insufficient attention is paid to the acid concentration of the final solution. The actual figures given by these authors are incomplete, but a rough approximation shows the concentration of sulphuric acid to be between 8 and 10 per cent. by vol. By keeping the acid concentration down to 5 per cent., or slightly less, better results were obtained, and the precipitates were contaminated by only very small quantities of iron.

Precipitation temperature.—The temperature of the solution at the time of

precipitation should be below 10° C., for above this temperature there is a danger of reducing sexivalent molybdenum before it is precipitated. Even at the lower temperatures it is advisable to add a little bromine water to minimise the risk of reduction.

Amount of reagent required.—About three times the theoretical amount, as determined by the relation $1Mo \equiv 3C_6H_5.CH(OH).C:(NOH).C_6H_5$, is necessary for complete precipitation.

Time taken for complete precipitation.—Experiments have shown that precipitation is complete almost as soon as the reagent is added, but it appears advisable to allow the solutions to stand for about 10 minutes with occasional stirring. If the precipitate is allowed to remain in contact with the solution for more than 30 minutes there is a tendency towards low results.

The reagent.—In the early stages of this work an alcoholic solution of α-benzoinmonoxime was employed, but it was subsequently found that solutions in acetone yielded equally satisfactory results, and this latter solution was therefore adopted as standard.

The published method,[2] previously cited, suggests the use of 1 g. samples, but in view of the high molybdenum-content of the oxide (mentioned under the sulphide method) samples of from 2 to 5 g. were employed. It was found that, for general use, samples of 3 g. were most satisfactory and convenient.

The method finally adopted was as follows:—Dissolve 3 g. of the sample in 35 ml. of nitro-sulphuric acid (made by adding 250 ml. of conc. sulphuric acid and 300 ml. of conc. nitric acid to 1500 ml. of distilled water) as in the estimation of silicon, evaporate the solution to dryness, and heat the residue at 250° to 300° C. for 10 minutes. In some instances it has been found that no fumes are evolved at this stage, but this in no way affects the results. (If more sulphuric acid is added and the final solution is diluted to adjust the acid concentration to 5 per cent., the resulting volume of solution proves rather unwieldy.) Allow the residue to cool somewhat, add 100 ml. of hot 5 per cent. sulphuric acid, and boil the solution until all salts have dissolved. With some cast irons containing chromium it is difficult to dissolve the salts, and boiling may have to be continued for as long as 10 minutes. Filter off graphite, silica, etc., and wash the residue with three 15-ml. portions of 5 per cent. sulphuric acid and then six times with hot water. To the filtrate add 10 to 15 ml. of sulphurous acid, and boil for about 10 minutes to expel sulphur dioxide. Dilute the solution to 250 ml., cool to a temperature of 5° to 10° C., add, with constant stirring, 10 ml. of the reagent (a 2 per cent. solution of α-benzoinmonoxime in acetone) followed by sufficient bromine water to colour the solution a pale yellow, and finally add a further 5 ml. of the reagent. Allow the solution to stand at 5° to 10° C. for 10 to 15 minutes with occasional stirring, add a little ashless paper-pulp, and filter through a No. 40 Whatman filter-paper. The precipitate is more readily filtered off on a filter-paper than on a paper-pad; if suction is applied to the latter type of filter, filtration almost ceases. Wash the precipitate well with a 1 per cent. solution of sulphuric acid containing 2·5 per cent. of the reagent at a temperature of 5° to 10° C. Dry the washed precipitate and carefully ignite at as low a temperature as possible until all the organic matter has been burnt off and then at a temperature not exceeding 500° C. On no account

should the temperature be allowed to rise above this limit, owing to the volatility of the molybdic oxide. This proves a frequent source of error. The molybdenum is finally weighed as molybdic oxide, which contains 66·67 per cent. Mo.

The method requires careful manipulation if correct results are to be obtained, and the conditions given should be strictly adhered to. With 3-gram samples it has been found that, with the above-mentioned acid concentration, the contamination by iron of the precipitates obtained is negligible for routine purposes, being consistently less than 1 mg. If desired, a re-precipitation may be made by dissolving the precipitate in sodium hydroxide solution and precipitating the molybdenum as lead molybdate, as described under the purification of ignited sulphide precipitates.

In determining small amounts of molybdenum (below 0·1 per cent.) some iron is invariably precipitated, and it is best to dissolve the ignited precipitate in sodium hydroxide solution, and then to acidify with sulphuric acid and determine the molybdenum colorimetrically by adding stannous chloride and potassium thiocyanate solutions.[7]

Subsequently, over 100 determinations were carried out by this method, and it was found that the lower the temperature at which precipitation is made, the less the contamination due to iron; if precipitations are made in an ice-bath, only the merest trace of iron is carried down (less than 0·1 mg.).

I wish to express my thanks to the Director and Council of the British Cast Iron Research Association for permission to publish this work.

APPENDIX

Samples examined	Modified lead molybdate method Mo Per Cent.	α-Benzoin-monoxime method with direct weighing as MoO_3 Mo Per Cent.	Benzoin-monoxime method with re-precipitation as $PbMoO_4$ Mo Per Cent.	Sulphide method Mo Per Cent.
A { B.C.S. sample K (Molybdenum cast iron) Ni, 1·73 per cent. Cr, 0·41 ,,	0·37	0·35	0·35	0·39
	0·38	0·36	0·34	0·39
	0·37	0·39	0·37	0·38
	0·34	0·36	0·36	—
B { U.S. Bureau of Standards Sample. Mo, 0·687 per cent.	0·70	0·70	0·70	—
	0·67	0·65	—	—
C { Crankshaft (Cast iron) Ni, 0·16 per cent. Cr, 0·53 ,,	0·89	0·87	0·87	0·86
	0·89	0·87	0·86	0·86
	0·89	0·89	0·86	0·87
	0·89	0·88	0·87	0·86
	0·88	—	—	—
	0·88	—	—	—

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A Colorimetric Method for the Determination of Traces of Phenol in Water

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IT has long been known that extremely minute quantities of phenolic substances may give rise to an "iodoform" taste in water which is subsequently purified by chlorination, and that over 10 p.p.m. of tar acids in a river water may prove toxic to fish-life.

Several colorimetric methods have therefore been proposed for the determination of phenols in water. Perhaps the best known of these is the method of Fox and Gauge,[1] which depends on the formation of a yellow or red dyestuff when phenol is coupled with diazotised sulphanilic acid. Other workers (*e.g.* Hinden-Splittgerber,[2] Hilpert and Gille,[3] Folpmers[4]) have employed similar methods, using *p*-nitraniline in place of sulphanilic acid. Baylis[5] has based a procedure on the Gibbs indophenol test,[6] in which the phenol is converted into a blue indophenol by reaction with 2:6-dibromo-quinonechloroimide. The Folin and Denis method[7] employs phosphotungstic-phosphomolybdic acid, which gives a blue colour with phenol, but it has been stated[8] that the test is not specific for aromatic compounds. The Millon reagent has also been employed for the detection of phenols (Bach[9]).

Probably the most satisfactory of these methods is that of Fox and Gauge, but the reagent gives a slight yellow colour with certain vegetable substances present in surface waters, and, being applied in alkaline solution, difficulties may arise through precipitation of calcium and magnesium salts. To overcome these objections the authors direct that the phenol should be extracted with chloroform, and washed therefrom with alkali, and the test applied to this alkaline solution. The Baylis method suffers from the disadvantage that the colour of the indophenol develops only slowly with small concentrations of phenol, and the test-solutions must be allowed to stand for several hours, or preferably overnight, before matching. There is also a sharply optimum *p*H range for maximum colour production, and sample and standard must be buffered so as not to differ from a *p*H of 9·6 by more than 0·2 unit. The sensitivity of the method is, however, great—about 0·01 p.p.m. for a direct test. A distillation technique is also described by which it is claimed that 0·001 p.p.m. of phenol may be determined, but, as Fox and Gauge[1] point out, distillation is open to the objection that some of the higher phenols are not volatile, while phenol itself only distils over slowly.

In an attempt to overcome these disadvantages, we have developed a new procedure in which the phenol is converted into an indophenol by the well-known method of oxidation with hypochlorite in the presence of dimethyl-p-phenylene-diamine. In this case the indophenols produced are readily soluble in organic solvents (unlike the indophenols produced by the Gibbs reagent) so that the sensitivity of the method may be increased by extracting the indophenol with carbon tetrachloride and matching the extracts. In tests on 200 ml. of a sample the sensitivity of the method is about 0·01 p.p.m. of phenol. The method has the advantage of quickness, and the pH of the solution is a far less critical factor than when the quinonechloroimide is used. Actually, satisfactory development of colour takes place provided that the pH is between approximately 7·0 and 8·5, and it is advisable to buffer both standard and sample with a small quantity of sodium bicarbonate before testing.

Table I shows the colour of the indophenol solutions obtained from a few of the higher phenols by the use of this method; the solutions tested contained 1 p.p.m. of the phenol in tap water.

TABLE I

Phenol	Aqueous solution of indophenol	Carbon tetrachloride solution of indophenol
Phenol	Blue	Purple
o-Cresol	Deep blue	,,
m-Cresol	,, ,,	,,
p-Cresol	No reaction	—
m-Xylenol	Pale blue	Blue
a-Naphthol	Purple	Purple
β-Naphthol	Very pale green	Green
o-Chlorophenol	Turquoise blue	Blue
p-Chlorophenol	Pale blue	Pale purple
Trichlorophenol	No reaction	—

As with the Gibbs reagent, p-cresol does not give the reaction.   The sensitivity of the test varies for different phenols, and for those tested it is greatest with o-cresol and least with β-naphthol. The only interfering substances so far encountered are the primary aromatic amines, which give coloured indamines with the reagent. Aniline, o-toluidine, m-toluidine, m-xylidine and the naphthylamines all react, but, with the exception of a-naphthylamine, the sensitivity is only about 1 p.p.m., which is much less than with the phenols. a-Naphthylamine may be recognised by the magenta-coloured solution which its indamine forms in carbon tetrachloride solution. Except in special cases, these amines are unlikely to be present in water unless accompanied by a large excess of phenol.

SOLUTIONS REQUIRED.—(i) p-Nitrosodimethylaniline, 0·1 per cent. in distilled water. The nitroso compound is dissolved by gradual addition to the water which is just below boiling-point; the solution is cooled and filtered, and will then keep for at least a week. The diamine reagent is prepared immediately before use by shaking the above-mentioned solution with a large excess of zinc dust until decolorisation is complete, the reduction being hastened by the addition of one drop of 10 per cent. copper sulphate solution for every 10 ml. of reagent. The

excess of zinc is filtered off, and the filtrate then usually exhibits a pale pink tinge which does not appear to be detrimental.

(ii) Sodium bicarbonate: a 5 per cent. aqueous solution.

(iii) Sodium hypochlorite solution containing 0·05 per cent. of available chlorine.*

(iv) Standard phenol solution: 1 ml. = 0·01 mg. of phenol. The solution should be prepared immediately before use by dilution of a 0·1 per cent. solution of phenol which has been standardised against 0·1 N bromide-bromate solution. In practice it has been found that the tint of the indophenols produced from polluted waters has generally been sufficiently near that of a phenol standard for satisfactory matching, but in certain cases it may be preferable to employ ortho- or meta-cresol or xylenol as standards.

METHOD.—The sample should be examined as soon as possible after collection, since dilute solutions of phenols are liable to undergo biological decomposition. It should be filtered if at all turbid.

(A) *When the phenol-content of the sample is greater than* 0·15 *p.p.m.*—It is always preferable to make a preliminary test in order to ascertain the approximate quantity of phenol present and the volume of hypochlorite solution which is required. To 100 ml. of the sample in a small flask are added 2 ml. of the bicarbonate solution, followed by 2 ml. of the filtered diamine reagent, and the hypochlorite is then run in gradually from a burette, with gentle agitation. The first addition of hypochlorite results in the formation of a pronounced pink colour, but this is completely discharged on adding more hypochlorite, and in the presence of over 0·10 p.p.m. of phenol the colour of the solution changes to pure blue, owing to the formation of indophenol. No further addition of hypochlorite should be made once the solution is free from any red tinge, since a large excess of chlorine would tend to bleach the blue colour.†

The approximate phenol-content of the sample is then determined by matching the blue colour obtained against the colour produced in a similar manner by a known volume of standard phenol solution, the matching being made in Nessler glasses by the balancing-column method.

To determine the phenol-content accurately, the sample is diluted so as to contain 0·15 to 0·30 p.p.m. of phenol and treated with diamine reagent and hypochlorite in the manner described above, and the blue colour is then matched exactly against one of a set of appropriate phenol standards. The colours are matched after standing for 2 to 3 minutes.

(B) *When the phenol-content is less than* 0·15 *p.p.m.*—The following procedure may also be employed when the sample is highly coloured. To 200 ml. of the sample in a separating funnel are added 4 ml. of the bicarbonate solution followed by 4 ml. of the diamine solution. The correct quantity of hypochlorite solution (already ascertained from a preliminary test) is then gradually run in, so as just to discharge the pink colour which first appears. The indophenol is now extracted

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* The hypochlorite employed in the present work was prepared from sodium carbonate and bleaching powder (sodium carbonate, 60 g.; bleaching powder, 40 g.; water, 400 ml.), the filtrate being diluted so as to contain 0·05 per cent. of available chlorine.

† It has been found that actually the titration with hypochlorite may be overdone by at least 0·5 ml. without materially affecting the results.

with two 10-ml. portions followed by one 5-ml. portion of pure carbon tetrachloride, and the extracts are dried by the addition of a little anhydrous sodium sulphate. The solution is finally extracted with a further 5 ml. of tetrachloride, which is also used to wash out any indophenol remaining in the sulphate used for drying the previous extracts.   The dry extracts are made up to a definite volume and matched in narrow Nessler glasses against standards prepared in precisely the same fashion. When the indophenol is extracted by this method care should be taken to ensure that sufficient hypochlorite has been added, since otherwise there is a risk of extracting incompletely chlorinated diamine, which is apt to impair the colour of the indophenol solution.

The following results were obtained when using the method for the determination of phenol which had been added to distilled water and to impure river water (hardness 280 p.p.m.: colour, 30 units, American Public Health Association).   The quantities of phenol added were unknown to the operator and visual matching was carried out in Nessler tubes.

TABLE II

Sample	Phenol added p.p.m.	Phenol found p.p.m.
River water	0·025	0·024
,,	0·065	0·068
,,	0·095	0·080
,,	0·135	0·14
,,	0·170	0·15
,,	0·33	0·31
,,	0·53	0·50
,,	0·67	0·64
,,	0·87	0·90
Distilled water	0·15	0·15
,,	0·30	0·30
,,	0·50	0·50
,,	0·73	0·73
,,	0·90	0·86

In conclusion, we wish to thank Mr. L. D. K. Morrison for his assistance during the course of this work, and the Chairman and Directors of the Southend Waterworks Company for their permission to publish the results.

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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.

AN UNUSUAL ADULTERANT FOR PEPPER

FROM one of our Authorities we recently received four sealed samples of pepper, which we will designate A, B, C and D; the last was described as "Pepper" and the others as "White Pepper." Microscopically, they appeared to be perfectly genuine; D contained more husk than the others.

The ash of these samples we found to be 4·29, 1·02, 0·98, and 4·70 per cent., respectively, and when re-carbonated they were 8·28, 1·24, 1·22, and 8·78 per cent.

We examined the ash of samples A and D, in both of which we found notable amounts of magnesia, which we determined quantitatively, and found in A 7 per cent. and in D 8 per cent. (calculated as $MgCO_3$).

The two samples were further examined and found to contain magnesium carbonate. This adulterant, in these amounts, is difficult of detection, as it is likely to be missed in the microscopical examination, and, unless the ash is re-carbonated, its amount might not arouse suspicion, particularly if the sample be described as "pepper."

The appearance of these two samples was excellent—that of white peppers in fact, but on removing the magnesium carbonate by dilute acids, the original pepper in D was seen to be distinctly inferior to that in A, as containing considerably more husk.

Not only does the addition of magnesium carbonate improve the appearance of the pepper, but its employment presents some temptation, as its cost is about one-third that of pepper.

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THE PHOSPHATASE TEST

WE have been carrying out the phosphatase test upon samples of pasteurised milk for two local authorities in London for the last six months.

So far as our results indicate, we have found the test extremely useful, not only in detecting gross errors in pasteurisation, but also in revealing small errors in technique—such as insufficient period of heating (less than half-an-hour) or heating slightly below 145° F. Recently we have had an interesting border-line case which clearly indicates the utility of the test in this direction. A particular sample gave a blue value of 3·3 Lovibond units and we reported that the results indicated a slight error in technique. The sanitary inspector, therefore, checked the temperature records of the particular dairy in question, and he was able to ascertain that on that particular day the temperature of pasteurisation was near 140° F. for a considerable time.

Regarding the method of carrying out the test, we have also noticed a number of points emphasised in the paper by Anderson, Herschdörfer and Neave (see p. 86). There is one point, however, about the "buffer substrate" which is worth noting. We have used, throughout, tablets supplied by the British Drug Houses, and have found them quite satisfactory. These tablets are particularly useful when one has to make a few tests intermittently, and not regularly from day to day, because a

fresh solution of the buffer substrate can be easily prepared in a few minutes by dissolving one tablet in 50 ml. of chloroform water.

Finally, we would refer to the confusion that seems, from our experience, to exist in some quarters concerning the use of this test as an index of the bacteriological condition of milk. Some appear to think that if a sample passes the phosphatase test, it must necessarily be bacterially clean. Whilst this is true in most instances, it is important to emphasise the point that the phosphatase test gives no indication of any bacterial contamination that milk may receive *after* pasteurisation. For example, if a sample has been correctly pasteurised and then put into dirty containers or has otherwise been handled in some unhygienic way, it will still pass the phosphatase test, so that one cannot rely wholly on this test by itself and a bacteriological examination becomes necessary to guard against this contingency. The two in combination—*i.e.* the phosphatase test and bacteriological examination—afford as effective a control of pasteurised milk supply as we seem likely to get at present.   F. W. EDWARDS
                                                                                              H. R. NANJI

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## A METHOD FOR THE ANALYSIS OF CARBONATE, HYDROXIDE CYANIDE MIXTURES*

THE results given below and the method of obtaining them may be of interest to analysts faced with similar problems the solution of which is perhaps at first sight not self-evident. The solution, of which an analysis was required, was a cadmium plating bath and contained cyanide, hydroxide, carbonate, cadmium and alkali metal "ion." The method adopted was as follows:

(a) Carbonate was determined by treating the solution with a solution of barium nitrate, filtering off the barium carbonate, washing, dissolving in a measured excess of standard hydrochloric acid, and titrating the excess. Addition of barium nitrate and the filtering and washing operations were carried out in an atmosphere free from carbon dioxide.

(b) Hydroxide was determined by treating the filtrate from (a) with a solution of silver nitrate and ammonium nitrate and then with a measured excess of standard sulphuric acid, filtering and washing, treating the filtrate with 2 to 3 g. of sodium chloride, again filtering and washing, and titrating the excess acid back with standard alkali, using methyl red as indicator.

(c) Cyanide was determined on a fresh portion of the sample gravimetrically by adding ammonium nitrate and silver nitrate, making faintly acid with nitric acid, filtering off the precipitated silver cyanide, washing, drying and weighing.

†(d) Alkalinity due to hydroxide and carbonate combined was determined by adding silver nitrate and ammonium nitrate, followed by a measured excess of standard sulphuric acid, filtering and washing, adding an excess of sodium chloride, again filtering and washing, and titrating back the excess acid, using methyl red as indicator.

†(e) Total alkalinity was determined by adding an excess of standard sulphuric acid, boiling, cooling and titrating back the excess acid.

(f) Cadmium was determined as usual by precipitation as sulphide and weighing as sulphate.

  * Communication from the Research Department, Woolwich.
  † Check determination.

The only part of the method which calls for comment is the precipitation, filtration and washing of the barium carbonate precipitate in (*a*). The apparatus used was the percolator already described in this journal (Evans, ANALYST, 1926, **51**, 230). A pulp filter was placed in the bottom of the main funnel B. The tube D was connected to a soda-lime tower and tube F to the pump, and a slow stream of air was drawn through until carbon dioxide had been removed from the apparatus. The pump was then detached and the tube F closed; the sample was run in through the top funnel E on to the filter (the tap G of the main funnel being closed) and rinsed in with a little water (care being taken not to admit any air); the barium nitrate solution was added in the same way. When precipitation was complete the tap G was opened and the solution allowed to filter into the flask; if there was any haze in the liquid (showing that barium carbonate had been carried through the filter), the filtrate was re-transferred to the main funnel by closing the tap G and re-attaching the pump to F, and was then re-filtered. When the filtrate was bright the precipitate was washed by admitting quantities of cold boiled-out water through the top funnel and allowing them to run through the filter. Solution of the barium carbonate was achieved by disconnecting the apparatus, closing the tap G, running the required amount of acid into the main funnel, stirring and digesting. The initial precipitation can, if required, be carried out in the flask, both sample and precipitant being filtered into it; in that case the liquid and precipitate are transferred to the top funnel, as outlined above, and the remains of the precipitate rinsed up with repeated filtrations and transferences. The tap H of the return tube was allowed to remain open throughout the operations.
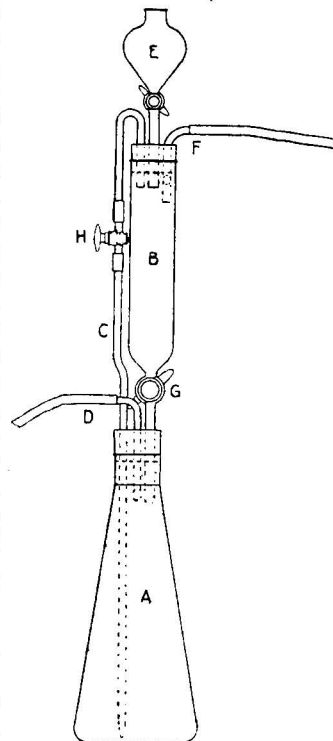
The results obtained were as follows:

		Grams per litre		NaOH equivalent
(*f*) Cd	.. ..	42·9		
(*c*) CN′	.. ..	48·4		
(*b*) OH′ expd. as NaOH	.. ..	21·2	≡	21·2
(*a*) CO″$_3$ expd. as Na$_2$CO$_3$	.. ..	90·0	≡	68·0

$$\overline{89·2}\begin{cases}\text{alkalinity calc.}\\ \text{from OH′} + \text{CO″}_3\end{cases}$$

(*d*) Alkalinity due to OH′ + CO″$_3$
    expd. as NaOH   ..   ..   —   =   89·0   (determined)

	Determined	Calculated from CN′+OH′+CO″$_3$
(*e*) Total alkalinity expd. as no. of ml. *N*/10 acid required for 1·00 ml. of sample	41·50	40·95

The calculated total alkalinity includes, of course, the combined errors on the three determinations.   B. S. EVANS

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THE DETERMINATION OF PHOSPHORUS IN SILVER PHOSPHATE

RECENTLY Bloom and McNabb[1] published a method for the volumetric determination of silver by titration with potassium iodide, using ceric ammonium sulphate and starch as indicators. This procedure has been extended to the determination

of phosphorus after the precipitation of silver phosphate and its subsequent solution in nitric acid. This method gives excellent results, but interfering ions, such as those of arsenate, tungsten, sexivalent chromium and molybdenum, must be absent. The use of the potassium iodide method is advantageous, since it eliminates frequent standardisations. The standard solution may be prepared by direct weighing.

In precipitating silver phosphate it is necessary to maintain the proper pH. Kolthoff[2] points out that good results may be obtained by precipitation of the silver phosphate in a buffered solution. He also states that if the silver phosphate is dissolved in nitric acid and the silver determined by the Volhard procedure, low results are obtained, owing to the loss of silver phosphate by washing. The procedure recommended is similar to that given by Kolthoff.[2] The precipitate was washed, however, with a saturated silver phosphate solution. Results were low unless this modification was employed.

Procedure.—Weighed samples of pure, dry potassium dihydrogen phosphate were dissolved in about 100 ml. of water. Two to three drops of methyl orange indicator were added. (If free acid is present it is necessary to add alkali.) A 5 to 10 ml. excess of 0·1 N silver nitrate solution was added with stirring, followed by the dropwise addition of 8 ml. of a saturated sodium acetate solution. After the solution had been filtered, the precipitate was washed by decantation with saturated silver phosphate solution until a portion of the filtrate gave only a faint turbidity when tested for silver ions. The precipitate was then dissolved off the filter with approximately 30 ml. of hot 2 N nitric acid, washed with hot water and collected in the original beaker. Sufficient 6 N sulphuric acid was added to make the solution about 1 to 2 N with respect to this acid, and then 3 drops of 0·1 N ceric ammonium sulphate solution and 5 ml. of 0·5 per cent. starch solution. The volume at this point was 125 to 165 ml. The silver was then titrated with 0·1 N potassium iodide solution to a permanent blue-green end-point. A blank test was made, omitting the silver nitrate. It required about 0·15 ml. of the potassium iodide solution.

The following results were obtained:

Potassium dihydrogen phosphate g.	Phosphoric anhydride calculated g.	Phosphoric anhydride found g.	Difference g.
0·2000	0·1043	0·1042	−0·0001
0·2015	0·1051	0·1050	−0·0001
0·2112	0·1102	0·1103	+0·0001
0·2020	0·1054	0·1052	−0·0002
0·2008	0·1050	0·1048	−0·0002
0·2027	0·1058	0·1056	−0·0002
0·1013	0·0529	0·0528	−0·0001

SUMMARY.—A satisfactory volumetric method for the determination of phosphorus in silver phosphate has been described. After solution of the silver phosphate in nitric acid, the silver is titrated with potassium iodide, ceric ion and starch being used as internal indicators.

Washing the silver phosphate precipitate with water yields low results. This may be avoided by washing with a saturated silver phosphate solution.

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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 would be submitted to the Publication Committee.

CITY OF BIRMINGHAM

REPORT OF THE CITY ANALYST FOR THE THIRD QUARTER, 1936

OF the 1295 samples submitted by the Food and Drug Inspectors, only one was bought formally.

WHITE CHOCOLATE.—Three samples of a new type of confectionery were taken. These were sold as "White Chocolate" and were composed of a mixture of cocoa butter, dried milk and sugar. True chocolate, of course, should contain cocoa powder and should be of a brown colour. The name "White Chocolate," is therefore, a contradiction in terms. The label of one of the samples gave the composition of the article, and no action was taken in this case. The labels on the other samples gave no indication of the constituents, and both the firms concerned were communicated with. As a result, the name of each article was changed to "Milky Block," which does not suggest any affinity with chocolate.

TABLE CREAM.—A sample, labelled "Thick Rich Table Cream," contained only 21 per cent. of fat, whereas ordinary dairy cream contains an average of about 50 per cent. The use of the adjective "Rich" in the description of this sample is certainly not justified, and the vendor agreed to leave it out in future.

H. H. BAGNALL

Official Appointments

THE Minister of Health has approved the following appointments:—

REGINALD FRANK WRIGHT as a Public Analyst for the County Borough of Brighton, in addition to Samuel Allinson Woodhead (November 10th, 1936).

WILLIAM WILDERS TAYLOR as a Public Analyst for the County Borough of Nottingham, from January 1st, 1937, in place of S. R. Trotman, retiring (December 4th, 1936).

REGINALD FRANK WRIGHT as a Public Analyst for the County of West Sussex, in addition to Samuel Allinson Woodhead (December 9th, 1936).

ALEXANDER HENRY MITCHELL MUTER as a Public Analyst for the County of the Parts of Kesteven (Lincolnshire), in place of B. Henry Gerrans (deceased) (December 12th, 1936).

THOMAS MCLACHLAN as a Public Analyst for the Borough of Chelsea, in place of B. Henry Gerrans (deceased) (December 17th, 1936).

WILLIAM WILDERS TAYLOR as a Public Analyst for the Borough of Newark-on-Trent, from January 1st, 1937, in place of S. R. Trotman, retiring (December 17th, 1936).

DANIEL DONALD MOIR as a Public Analyst for the County of Surrey, in place of T. W. Glass, who has resigned, and in addition to Edward Hinks (December 30th, 1936).

Ministry of Health

BACTERIOLOGICAL TESTS FOR GRADED MILK*

STANDARDS.—1. The following bacteriological standards for graded milks are prescribed by the Milk (Special Designations) Order, 1936:

Tuberculin Tested Milk including *Tuberculin Tested Milk (Certified)* : *Accredited Milk.*—The milk when tested in accordance with the prescribed method must not decolorise methylene blue within $4\frac{1}{2}$ hours if the sample is taken at any time from the 1st May to the 31st October; or within $5\frac{1}{2}$ hours if the sample is taken at any time from the 1st November to the 30th April. The milk also must not contain coliform bacillus in 1/100 millilitre.†

Tuberculin Tested Milk (Pasteurised).—The milk must not contain more than 30,000 bacteria per millilitre.

Pasteurised Milk.—The milk must not contain more than 100,000 bacteria per millilitre.

SAMPLING.—Pars. 2 and 3 and 5 deal with the collection of samples, and Par. 4 with sampling from churns.

TRANSPORT AND STORAGE OF SAMPLES.—Directions for Tuberculin Tested and Accredited Milks are given in Pars. 8 to 10 and for Pasteurised Milk and Tuberculin Tested Milk (Pasteurised) for the Plate Count Test in Par. 11.

TECHNIQUE OF THE TESTS.—Section A deals with the apparatus and technique of the Methylene Blue Test. Stress is laid upon the point that the reliability of the results of the test depends upon the strict observance of the directions given in the Memo. (Pars. 13–26). Standard methylene blue tablets must be used for the test. Traces of impurities normally present in methylene blue seriously affect the reliability of the test. It is essential, therefore, that only specially prepared standard methylene blue tablets should be used for this test. The names of manufacturers who supply such tablets may be obtained on application to the Ministry of Health.

The test tubes used must conform to the British Standard Specification 152/16, nominal 6″ × $\frac{5}{8}$″, having an internal diameter of $13·5 \pm 0·5$ mm. and an etched mark indicating 10 ml. One-ml. pipettes, preferably conforming to specified dimensions, are to be used, and no pipette should have an error in delivery of more than ± 2 per cent.

The milk is to be regarded as decolorised when the whole column of milk is completely decolorised or is completely decolorised up to within 5 mm. of the surface.

Section B (Pars. 27 to 34) deals with the Apparatus and Technique for the Coliform Test for Tuberculin Tested and Accredited Milks. The milk is regarded as satisfactory if two out of three tubes are found to be free from acid plus gas after 48 hours' incubation at 37° C.

Section C describes the Plate Count Test for Tuberculin Tested Milk (Pasteurised) and for Pasteurised Milk (Pars. 35 to 45), and the final paragraph (46) states that the results of the three tests should be recorded on forms similar to those given in the Appendix to the Memorandum, and that the report should be sent to the Licensing Authority immediately on the completion of the examination.

* *Memo.* 139/Food (Jan. 1937). For official use.

† It is not necessary that every sample should be submitted to both tests, and Licensing Authorities which require to have frequent tests made of samples of producers' milk may find it convenient to have most of the samples examined by the methylene blue test alone, reserving the coliform test for occasional use.

SALE OF FOODS AND DRUGS

EXTRACTS FROM THE ANNUAL REPORT FOR 1935–36 AND ABSTRACT OF REPORTS
OF PUBLIC ANALYSTS FOR THE YEAR 1935*

THE samples of foods and drugs analysed by Public Analysts in England and Wales during 1935 numbered 143,831 (an increase of 3248 on the previous year), and the number reported against was 7972, or a proportion of 5·5 per cent. against 5·3 for 1934 (ANALYST, 1935, **60**, 821).

PRESERVATIVES.—Of a total of 422 contraventions of the Regulations, 207 related to sausages, mostly on account of no declaration of the presence of the allowed proportion of sulphur dioxide. Boron preservative was found in cream, milk, butter, sausages, potted meats, etc.; formaldehyde in milk and in 2 samples of tinned smoked salmon, and sulphur dioxide in barley, minced, potted and other meats, pepper and vinegar. One minced meat contained 1860 p.p.m. of sulphur dioxide.

MILK.—The number of samples examined (78,674) shows a marked increase over the previous year, and of these, 5798 (7·4 per cent.) were reported adulterated or not up to standard. Of the 1048 "appeal to cow" samples, 406 were below the presumptive standard. Convictions were obtained in respect of a milk containing 24·7 per cent. of added water and of 4 milks with 19·2 to 39·6 of added water. Twenty samples contained added colouring matter, 8 samples boron preservative, and 11 formaldehyde. Of the 27 samples of skimmed or separated milks reported against, one contained colouring matter, one was stated to be whole milk, but in most cases there was a deficiency in non-fatty solids. Legal proceedings were instituted in respect of most of the samples found to contain preservative, and in one case in which the sample contained 2 p.p.m. of formaldehyde the producer was fined £25 and £1 11s. 6d. costs. Twenty-one samples (of 1186) of condensed milk were deficient in milk-solids, were unfit for human consumption, or contained the equivalent of less milk than was indicated on the label. Three dried milks (of 186) were unsatisfactory owing to deficiency of milk fat or contamination with foreign matter. One sample consisted of starch.

CREAM.—Fifteen of 1971 samples of cream were reported against, and 8 were deficient in fat. One "thick cream" contained 20·25 per cent. of fat, and two samples of tinned cream, labelled "Pure Thick Cream, a highly concentrated and rich cream," contained 18·5 and 20 per cent. of fat respectively.

BUTTER AND MARGARINE.—Of 8001 samples of butter and 2774 of margarine, 64 of butter and 7 of margarine had an excess of water; 7 butters contained boron preservative; 10 consisted wholly or partly of margarine; 4 contained excess of free fatty acids, and one was mouldy and dirty, whilst 45 margarines infringed the labelling regulations and 2 contained excess of milk-fat.

LARD AND OTHER FATS.—As many as 40 of 2688 samples of lard had unfavourable reports, mostly for containing foreign fats or oils. Of the 512 suets, 41 were reported against; undeclared or excessive starchy matter was present in most, whilst 3 contained excess of water and 3 gave evidence of decomposition.

CHEESE.—There were 24 adverse reports on 1458 samples examined; 4 samples were contaminated with tin and 2 with lead.

BREAD AND FLOUR.—Only one bread (containing fungus) of 264 examined was reported against and 6 of 1351 flours, one, sold as self-raising flour, being plain flour.

JAM AND MARMALADE.—There were 126 adverse reports on 1624 samples; 40 jams contained excess of sulphur dioxide, and one was also contaminated with copper and was deficient in fruit. One sample was heavily contaminated with mould spores; a "home-made" marmalade was factory-made, and most of the

* Published by H.M. Stationery Office, Adastral House, Kingsway, W.C.2.   Price 3d. net.

remaining samples were deficient in fruit or soluble solids, or infringed the labelling regulations.

VINEGAR.—Of 1949 samples examined, 148 were deficient in acetic acid, 10 contained preservative, 8 contained vinegar eel-worms, one was contaminated with mouse droppings, and one contained excessive lead. In others, artificial vinegar was present in samples sold as "Malt" or "Table" vinegars.

The vendor of a sample of "table" vinegar, which proved to be artificial vinegar and to be 46 per cent. deficient in acetic acid, was fined £5 and 10s. 6d. costs.

SPIRITS AND BEER.—In 57 samples of whiskey, 26 of gin, 24 of rum, and 5 of brandy (from a total of 1706), the spirit had been reduced more than 35° under proof. Seven of 425 samples of beer were adulterated; 2 contained excessive amounts of sulphur dioxide, 3 excessive arsenic. A sample of ale contained at least 0·0003 per cent. of phenol, and a milk stout containing 5 per cent. of milk-sugar was reported against, as its description containing the words "Pure Dairy Milk" was misleading.

MISCELLANEOUS ARTICLES OF FOOD.—The 32,000 samples examined covered a very wide range and included potted meat containing excess of water, or starchy matter, or sulphur dioxide, or boric acid; sausages deficient in meat; fish cakes containing no fish; several samples of tinned fish contaminated with tin, involving destruction of stocks. Copper was present in tomato soup and catsup; copper and tin in tomato purée; copper and zinc in aerated water; copper and iron in frozen lambs' tongues and livers; zinc in gravy browning, in the liquor of pickled cucumbers, and to the extent of 640 p.p.m. in black treacle. Barleys and rices contained talc or other facing material; a ground rice contained a large admixture of maize flour similar to hominy, and tapiocas were sold as sagos. Samples of cinnamon, dried herbs, etc. contained sand, and some samples of dried mint, ailanthus leaves; baking powders were deficient in available carbon dioxide. The word "cream" in cake and pastry descriptions was responsible for a number of adverse reports. A wine contained 0·3 per cent. of linseed oil and traces of paraffin, and a "Ruby Fancy Tawny Wine" was a flavoured and coloured sugar solution.

A few samples of sugar contained sand, one contained 1·5 per cent. of salt, and some contained excessive quantities of sulphur dioxide.

DRUGS.—Of 6188 samples of drugs, 353 were adulterated or not up to standard. Various halibut oils were deficient in halibut oil, 3 cod-liver oils did not satisfy the B.P. antimony trichloride test, and 2 samples of "Malt Halibut Liver Oil" caramels, labelled as containing vitamins A and D, showed no evidence of the presence of any vitamins. A sample of orange quinine wine contained 800 p.p.m. of benzoic acid and no wine, and another sample consisted of a solution of quinine salt, sweetened with sugar, coloured and flavoured. Zinc, boric, carbolic, calomel and mercury ointments were amongst the samples reported against. Sweet spirits of nitre samples were deficient in ethyl nitrite, and solutions of iodine were deficient in, or contained excess of iodine or potassium iodide, or both. Some iodine ointments were more than 90 per cent. deficient in iodine. Talc was reported in aspirin tablets, and arsenic in tartrated iron.   D. G. H.

Home Office

Statutory Rules and Orders. 1936. No. 686

FACTORY AND WORKSHOP. NOTIFICATION OF DISEASES (MANGANESE POISONING)

THIS Order,* dated June 29th, 1936, made by the Secretary of State, extends the provisions of Section 73 of the Factory and Workshop Act, 1901 (1 Edw. 7, c. 22), to manganese poisoning. It came into force on August 1st, 1936.

British Standards Institution

BRITISH STANDARD SPECIFICATION

No. 718—1936. NEW BRITISH STANDARD FOR DENSITY HYDROMETERS

THE Committee entrusted with the work came to the conclusion that any attempt to standardise hydrometers type by type would tend towards perpetuating a state of quite unnecessary confusion rather than towards achieving simplicity and uniformity. They were, therefore, driven to consider the whole question of hydrometers and hydrometry from first principles.

As a result, their activities were concentrated on providing a standard specification for density hydrometers and on compiling appropriate tables for use with such hydrometers.

The hydrometers are adjusted to indicate density—mass per unit volume—in grams per ml. at 20° C. The basis of the scale is thus entirely free from ambiguity and is expressed in universally recognised units. A wide choice of hydrometers is provided of varying range and accuracy and The National Physical Laboratory has made arrangements for testing hydrometers for conformity with the specification.

The hydrometers have been designed so that changes in reading due to changes in surface tension are as small as practicable. The surface tension for which each hydrometer shall be adjusted is specified and simple correction tables are given for use when the hydrometers are read in liquids having surface tensions different from those specified should the accuracy desired render the application of these corrections necessary. Temperature correction tables and tables for use in the measurement of liquid in bulk are also included in the specification.

Tables giving, over a temperature range, generally from 10° C. to 40° C., the density and composition of a number of liquids including sugar solutions, sodium chloride solutions, caustic soda solutions, sulphuric acid, hydrochloric acid and nitric acid are in course of preparation and will be published separately. The tables correlate density and percentage composition and so can be used not only in conjunction with the standard hydrometers, but also with any other method of determining density.

The Committee will willingly consider suggestions for the preparation of tables for other liquids of industrial importance, and every endeavour will be made to provide any table considered to be of use to industry.

Copies of this Specification (B.S.S. No. 718) may be obtained from the British Standards Institution, 28 Victoria Street, London, S.W.1. Price 3s. 6d. (3s. 8d. post free in Great Britain and Ireland, 3s. 10d. post free overseas).

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* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 1d. net.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Fat and Phosphatide-content of Wheat Germ. B. Rewald. (*Chem. and Ind.*, 1936, **55**, 1002–1003.)—The phosphatide-content of wheat germ (which is higher than that of other parts of the kernel) is important in bread-making, since, after removal of the germ, phosphatides in small amounts are added to bring up the phosphorus-content of the treated flour to that of the original grain. Determinations of the fat and phosphatides were made by the following method, found applicable to other natural products, such as egg, soya bean and animal tissues:— The germ is extracted with petroleum spirit, which extracts most of the fat and all those phosphatides which are in the so-called "free" state, *i.e.* not combined with proteins or carbohydrates. The extraction is continued until no more fat or lipoid substance is obtained. After removal of the solvent, a pale yellow substance remains. This is Extract No. I. The residue is dried until free from solvent and re-extracted with a boiling mixture of 80 parts of benzene and 20 parts of alcohol until exhausted. Sometimes more than twenty extractions are necessary. The extract, after removal of the solvents, is dark brown and contains fat, phosphatides and other substances. This is Extract No. II. By adding acetone to Extract No. I (which contains only fat and phosphatides) the fat is dissolved, and the insoluble phosphatides are washed with acetone until free from fat. The fat recovered from solution is very pale in colour, contains 11·2 per cent. of free fatty acids, is easily purified and can be used as a high-grade oil for nutritive purposes. The precipitated phosphatides, consisting of lecithin and cephalin, are separated by means of cold methyl alcohol, in which only the lecithin is soluble. The isolated lecithin (which contains 3·53 per cent. of phosphorus) is of a pale yellow colour and crystalline; the cephalin (containing 4·24 per cent. of phosphorus) is of a darker colour and crystalline. Extract II is dissolved in benzene to separate carbohydrates, proteins, etc., which are not soluble in this solvent, and the solution is treated with acetone to precipitate phosphatides and separate the fat. The fat is a dark brown oil containing about 25 per cent. of free fatty acids. The phosphatide fraction of this extract is of a dark colour. To separate carbohydrates and other substances still present, the phosphatide fraction is brought into colloidal solution in water, and the solution is extracted with ether, which extracts the phosphatides, leaving the other substances in the aqueous layer. As before, the lecithin and cephalin are separated by means of methyl alcohol. The phosphorus-content of the lecithin fraction thus separated was 3·49 and that of the cephalin fraction 4·94. The isolated sugar was a polysaccharide which could not be crystallised and which reduced Fehling's solution only after hydrolysis. The results obtained were as follows:—Extract I:—oil, 6·7 per cent.; lecithin, 0·181 per cent.; cephalin, 0·042 per cent. Extract II:—oil, 0·85 per cent.; lecithin,

0·31 per cent.; cephalin, 0·073 per cent. The total amount of phosphatides in wheat germ is thus 0·606 per cent., of which 63 per cent. is "bound." Of the total phosphatides, 80 per cent. is of the lecithin type and 20 per cent. of the cephalin type. The total amount of fat extracted was 7·55 per cent., but it is noteworthy that only 6·7 per cent. was extractable by petroleum spirit.   A. O. J.

Determination of the Maturity of Canned Peas. V. B. Bonney and S. C. Rowe. (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 607–619.)—The peas employed in the experiments, after being graded, blanched in boiling water and washed in cold water, were canned in brine containing 15 lb. of salt and 25 lb. of sugar to 100 gall. of water. The results of the following tests are tabulated in correlation with the district of cultivation, grade, variety and state of maturity of the peas. (1) *Drained weight.*—The contents of a can are transferred to a dish, mixed with twice their volume of water and poured on to an 8-mesh screen of diameter 8 inches and spread evenly over the surface. The screen is tilted as far as is possible without disturbing the peas and is allowed to drain for 2 minutes. The surplus water is removed by wiping the under surface of the screen with a cloth, and the peas are then transferred to a tared dish and weighed. (2) *Number ruptured over 1/16th inch.*—In 100 drained peas the number having the skin broken open by internal pressure to such an extent as to separate the broken edges more than 1/16th in. is counted. (3) *Number sinking in brine of sp.gr.* 1·12 (68° F.).—A weighed amount of drained peas is poured into this standard brine in a vessel which ensures that the brine is a little over 4 in. deep, and that the floating peas form a single layer. After 15 seconds the floating peas are skimmed off, drained by the method already described, and weighed. The percentage weight sinking in the brine is calculated. (4) *Solids insoluble in alcohol.*—A portion of the sample of drained peas is ground in a food chopper and stirred until homogeneous, and 20 g. are weighed into a 600-ml. beaker. After 300 ml. of alcohol (80 per cent. by vol.) have been added, the beaker is covered and the contents are heated to boiling point, allowed to simmer for 30 minutes and then filtered by suction through a dry tared paper on a Buchner funnel. The residue on the paper is washed with 80 per cent. alcohol until the washings are clear and colourless. The filter, with the solid matter, is dried at 100° C. for 2 hours, then in a desiccator, and finally weighed. A study of 157 samples of Alaska peas and 159 samples comprising 8 sweet varieties of peas showed that the figure for alcohol-insoluble solids provides an excellent criterion of maturity. With the exception of one sample (which gave the figure 22·8) the alcohol-insoluble solids were below 22 per cent. in all samples of Alaska peas which were not fully mature. The corresponding figure for sweet varieties of peas was 19 per cent. No samples of Alaska peas which were not packed from mature peas were found with over 5 per cent. showing a 1/16th in. rupture. Similarly, no peas which were not packed from mature peas showed over 17 per cent. sinking in the standard brine. Sweet varieties of peas, even when mature, are not appreciably ruptured when canned, and, since their sp.gr. is less than that of Alaska peas, very few sink in the standard brine. The effect of varying the composition of the brine was not investigated.   A. O. J.

Determination of the Maturity of Canned Stringless [Snap] Beans.
S. C. Rowe and V. B. Bonney. (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 620–628.)—
Three degrees of maturity are defined, *viz.*:—(1) *Tender*: small or medium-sized
pods which snap readily when bent, the seeds being undeveloped in the pod;
(2) *Fairly tender*: large pods which snap when bent, the seeds being well developed
but tender; (3) *Distinctly mature*: large pods, tough, leathery, rubbery or limp,
which no longer snap when bent, the seeds being generally well developed but
slightly shrivelled. The beans were sorted into various stages of maturity and,
after the ends had been removed by hand, were cut into inch-lengths and blanched
in boiling water. The cans were then filled as full as possible by hand, and the
interstices were filled with factory brine (2 to 3 per cent. of salt). Preliminary
experiments proved that the amount of tough fibrous material in the pods and the
percentage of seeds present are of value as criteria of the state of maturity of the
beans. The methods employed were as follows:—(1) *Drained weight*.—The contents
of a can are mixed with twice their volume of water and poured on to an 8-mesh
screen. For cans containing less than 3 lb. net weight an 8-inch screen is used;
for larger cans a 12-inch screen. The drained weight is then determined as described
in the previous abstract. (2) *Weight of seeds*.—The seeds from 150 g. of drained
material are separated and weighed. (3) *Fibrous matter*.—The drained beans
(100 g.) separated from the seeds are cut into half-inch lengths, pulped in a mortar,
transferred to the metal cup of a malted milk mixer with 200 ml. of boiling water
and boiled for exactly five minutes with 25 ml. of 50 per cent. sodium hydroxide
solution after the addition of a small piece of paraffin wax. The mixture is then
stirred for exactly 5 minutes by means of a malted-milk stirrer (capable of a no-
load speed of at least 7200 rev. per min.), and is filtered by suction through a monel
metal 30-mesh screen fitted into a Buchner funnel, the pulp being washed with a
$\frac{1}{4}$-inch stream of boiling water until free from alkali and again with a stream of
boiling water until the pulp is removed and the washings are clear. The residual
fibre is dried for 2 hours at 100° C. and weighed. (4) *Solids insoluble in alcohol*.—
The remainder of the drained material is treated as described in the previous
abstract. The beans investigated were graded by expert graders into 4 grades,
viz.: (i) fancy, (ii) extra-standard, (iii) standard and (iv) sub-standard. The
maximum limit of fibrous matter for grade (iii) was 0·08 per cent. With a single
exception, the maximum seed figure for grade (iii) was 6 per cent. The maximum
weight of solids insoluble in alcohol for grade (iii) was 7 per cent., but this figure is
not as diagnostic as the amounts of seed and fibre. Many of the samples regarded
by the graders as sub-standard contained an excessive amount of "strings" (par-
ticularly the variety Kentucky Wonder), but, even where there was objectionable
string development, the pods did not contain sufficient fibre to render the product
unsatisfactory. Toughness of string was measured as follows:—A string is
removed from the pod and one end is immediately fastened to a clamp weighing
$\frac{1}{2}$ lb. The string is held in the fingers at a distance of $\frac{1}{2}$ inch from the clamp, and
the weight is gently lifted. If the string supports the weight for at least 5 seconds,
it is regarded as a tough string. Examination of the majority of the 104 samples
for tough strings indicated that there should not be more than one tough string
for each 2 oz. of drained material.   A. O. J.

Determination of Reducing Sugars by the Rosenthaler-Curli Method.
Y. Volmar and S. Klein. (*J. Pharm. Chim.*, 1936, **24**, 400–409.)—Methods for
the determination of reducing sugars which depend on the use of a cupro-potassium
reagent, with determination either of the quantity of cupric hydroxide necessary
to oxidise the sugar, or of the quantity of cuprous oxide formed, have the dis-
advantages that elaborate precautions must be taken to prevent the re-oxidation
of the cuprous oxide, and that the reduction may proceed too far, giving a pre-
cipitate which is a mixture of cuprous oxide, cuprous hydroxide and copper. The
method studied by the authors is that of Rosenthaler (*Z. anal. Chem.*, 1904, **43**,
282; Abst., ANALYST, 1904, **29**, 209), Lehmann (*Arch. Hyg.*, 1898, **30**, 274) and Curli
(*Bull. della Industria delle Conserve Alimentari*, 1934, **9**, 100), depending on the
reduction of alkalinity which occurs during the oxidation of sugars by a cupric
salt. The reduction of alkalinity is due to the formation of acids by oxidation
of the aldehyde group of the sugars, and the conversion of cupric hydroxide in the
cupro-potassium complex by the acids into insoluble cuprous oxide or hydroxide.
The diminution in each reaction is directly proportional to the quantity of sugar.
The reagents used are: (*a*) a cupric solution, containing 69·28 g. of pure crystalline
copper sulphate in 1 litre of solution; (*b*) a glycerin solution, prepared by mixing
4 parts of pure glycerin with 1 part of boiled distilled water (the authors used twice
distilled glycerin, as pure commercial glycerin always gave high results, but if the
glycerin solution is made up with 70 per cent. alcohol instead of water, the
results are the same as those obtained with twice distilled glycerin if the quantity
of sugar to be determined exceeds 0·04 g.); (*c*) an *N*-solution of sodium or
potassium hydroxide; (*d*) an *N*/2-solution of sulphuric acid, standardised against
solution (*c*); (*e*) an indicator sensitive to organic acids, such as a 0·1 per cent. solution
of Congo red or haematin, a 0·2 per cent. solution of methyl red, or a fluorescent
indicator, such as umbelliferone. The method is as follows:—Thirty ml. of
solution (*a*), 20 ml. of solution (*b*), 40 ml. of solution (*c*) and 50 ml. of boiled distilled
water are mixed in a flask, heated to boiling, and treated gradually with 25 ml. of
the sugar solution, which must contain less than 1 per cent. of sugar. The boiling
is continued for about 2 minutes, and 50 ml. of hot boiled distilled water are then
added. After cooling, the volume is made up to 250 ml., and the cuprous oxide is
allowed to settle. A volume of 125 ml. is filtered through paper or asbestos, and
the excess of alkali is titrated. A blank was carried out when very dilute sugar
solutions were used, 25 ml. of boiled distilled water being added instead of the
sugar solution. The quantity of sugar (*M*) is given by $M = K(40 - n)$, where n
is the number of ml. of *N*/2 acid solution used, and K is a constant. This method
was tested on solutions of glucose and other hexoses and pentoses (arabinose,
laevulose, galactose, and invert sugar) of varying strengths from 0·02 to 1 per
cent., and the values for the diminution of alkalinity N ($= 40 - n$) were deter-
mined. Up to 0·5 per cent. the diminution of alkalinity is exactly proportional
to the quantity of sugar, the value of K in the above equation being 1·23. With
more concentrated solutions the results obtained are slightly too low. The
method can be applied to the determination of sucrose (which must previously be
inverted by boiling with a drop of hydrochloric acid, followed by careful neutralisa-
tion of the liquid), lactose, and maltose, proportionality being exact for solutions
of strengths up to 0·5 per cent.; K for lactose is 1·8, and for maltose 2·2.   E. M. P.

**Determination of Neutral Esters in Fermentation Liquids.   L. Espil
and E. Peynaud.** (*Bull. Soc. Chim.*, 1936, 5th Series, **3**, 2324–2325.)—The
method previously described (*id.*, 1935, **2**, 682) is suitable for simple solutions of
neutral esters, but with other products (*e.g.* wine) the ethereal extract of the esters
is coloured, indicating the presence of other substances, extraction at pH 7 is
incomplete even after long periods, and there is evidence of the presence of acid in
the extract.   Petroleum spirit (sp.gr. 0·650, b.p. 45° to 70° C.), however, gives
with such samples extraction-curves which tend rapidly to a limiting value and
are similar in shape to those obtained with ether and simple solutions.   In practice
the solvent is distilled in such a way that it is condensed in fine droplets which
pass through the sample under examination, so removing the esters and sub-
sequently falling into a lower vessel containing a standard solution of sodium
hydroxide free from carbonate; saponification then occurs, the residual alkalinity
being determined when extraction is complete.   The operation is continuous, and
50 ml. of wine may be treated in about 10 hours.   Volatile acids in the alkaline
liquid may be determined by acidification and distillation, the sum of the malic,
tartaric and citric acids being obtained by precipitation as barium salts in 70 per
cent. alcohol, and manganimetric titration.   Data for a red Burgundy (pasteurised
in 1893, the year of vintage), St. Emilion (1914), Sauterne and Medoc types (1923
to 1934), and Quinsac (1936) are, in milli-equivalents per litre:—Total acidity,
81, 89, 89 to 104, and 90; volatile acidity, 29·4, 19·4, 16·9 to 20·0, and 11·2;
neutral esters of volatile acids, 3·1, 2·0, 1·65 to 1·9 and 1·0; neutral esters
of tartaric, malic and citric acids, 0·5, 0·65, 0·5 to 0·95 and 0; neutral esters of
lactic and succinic acids, etc., 2·1, 1·25, 0·85 to 2·3 and 0·5; pH value, 3·94, 3·50,
2·94 to 3·20 and 3·60; alcohol, 15·1, 11·1, 9·4 to 13·2 and 10·6 per cent. by volume.

<div style="text-align: right">J. G.</div>

**Detection of Ferrocyanide Fining in Wines. L. Chauveau and A.
Vasseur.** (*Ann. Falsif.*, 1936, **29**, 470–472.)—The following modification of
Braun's reaction (formation of potassium isopurpurate) has been found specific
for the detection of ferrocyanide fining in wines.   Four to twenty ml. of the wine
are evaporated nearly to dryness after being rendered alkaline by a slight excess of
sodium hydroxide.   The residue is dissolved in a little warm water, and placed
in a 100-ml. Erlenmeyer flask, a crystal of tartaric acid is added, and a strip of
sodium picrate paper is inserted and held by the cork.   On warming the flask on
a water-bath the presence of cyanogen compounds is disclosed by the paper
becoming orange-red in less than 20 minutes.   Three hundredths of a mg. of
hydrocyanic acid in a sample of 20 ml. may thus be detected.   No advantage is
gained by taking a larger sample of wine.   The last traces of Prussian blue are
precipitated very slowly from wines treated with ferrocyanide, and the deposit
should always be examined by decomposing it with soda and treating it as described
above.   A positive reaction may then be obtained, even when the supernatant
liquid has given a negative one.   Also, a turbid sample of well-shaken wine may
be tested, and ferrocyanide then detected in wines containing an appreciable
amount of iron.

<div style="text-align: right">D. G. H.</div>

Occurrence and Possible Significance of some of the Minor Component Acids of Cow Milk Fat. T. P. Hilditch and H. Paul. (*Biochem. J.*, 1936, **30**, 1905–1914.)—Detailed analyses were made of the component acids of typical cows' milk fat, and the results were calculated (*a*) on the assumption that no unsaturated acids having a molecular weight lower than that of oleic acid were present, and (*b*) allowing for the presence of decenoic, tetra- and hexa-decenoic acids on the basis of special analyses made to determine the saturated ester-contents of the ester fractions which were obtained by the fractional distillation of the "liquid" methyl esters. When these results were compared with those obtained by the similar analysis of the component acids of the completely hydrogenated butter, it was found that the total amounts of the C_{16} and C_{18} acids present agreed only when the allowance was made, as in (*b*), for the presence of lower unsaturated acids. In addition to the traces of decenoic acid and about 1 per cent. of tetra-decenoic acid which was reported by Bosworth and Brown (*J. Biol. Chem.*, 1933, **103**, 115), the authors conclude that about 4 to 5 per cent. of hexa-decenoic acid must also be present. When this correction was applied, the amount of palmitic acid was increased by about 2 per cent., and the amount of oleic acid was reduced by about 5 per cent. No unsaturated acid having a molecular weight lower than that of $\Delta^{9\,:\,10}$ decenoic acid was detected, and the position of the double bond relative to the carboxyl group was found to be the same in the decenoic, tetra-, hexa-, and octa-decenoic acids of the butter-fat. It is suggested that these observations are in agreement with the hypothesis, put forward in earlier communications, that the lower saturated glycerides of milk-fats have been produced from pre-formed oleo-glycerides, and that these minor, lower unsaturated components may represent degradation products of oleo-glycerides which have escaped complete saturation to lower saturated groups. S. G. S.

Higher Saturated Fatty Acids of Butter-fat. G. E. Helz and A. W. Bosworth. (*J. Biol. Chem.*, 1936, **116**, 203–208.)—Hexacosanoic (cerotic) acid has been isolated from butter-fat. This acid, which crystallises from acetone as nacreous plates, has m.p. 80·5° C., and forms a methyl ester which distils at 286° C. at 15 ± 0·1 mm. pressure and at 261° C. at 5 ± 0·1 mm. pressure. The m.p. of the methyl ester is 62° C., and that of the amide is 105–107° C. In the butter investigated a saturated fatty acid having a molecular weight higher than that of stearic acid was found and the available evidence suggests that this is hexacosanoic acid. S. G. S.

Presence of Hydrocarbons in Arachis Oil. H. Marcelet. (*Bull. Soc. Chim.*, 1936, [5], **3**, 2055–2057.)—An investigation similar to that on olive oil (ANALYST, 1936, **61**, 488, 772). The residue from the deodorisation process contained a large proportion of mechanically entrained oil and, when freed from water, showed practically the same properties, with the exceptions of acidity and unsaponifiable matter, as did the oil itself. The unsaponifiable matter was somewhat sticky, and on crystallisation from alcohol yielded large crystals which showed the characteristics of phytosterol, and an oily, coloured liquid. The isolation of this oil was accomplished by distilling under vacuum the residue from the refining of the arachis oil, followed by saponification of the distillate and washing with petroleum

spirit.   The amber-coloured, unpleasant smelling oil had a sp.gr. 0·8020 at 19° C. and n_D^{19} 1·4518.   This oil was separated by distillation under a pressure of 3 mm. of mercury into three fractions, the first of which was too small in quantity for examination; the second and third fractions had the properties shown in the table.

	Fraction II	Fraction III
Boiling-point at 3 mm. pressure, °C. ..	120–125	180–185
Sp.gr. at 15°/15° C.	0·8200	0·8550
n_D^{19}	1·4650	1·4761
Iodine value (Hanus)	121	78
Molecular weight (by cryoscopic method)	209	267

Elementary analysis

Fraction II. $C_{15}H_{30}$ Found   C 86·06   H 14·40
Theoretical   C 85·71   H 14·28
Theoretical molecular weight 210.

Fraction III. $C_{19}H_{38}$ Found   C 85·52   H 14·45
Theoretical   C 85·71   H 14·28
Theoretical molecular weight 266.

These two hydrocarbons are related to hypogaeic and arachidic acids and have been named "hypogene" and "arachidene" respectively.   E. M. P.

Shea Nuts from the Gold Coast.   (*Bull. Imp. Inst.*, 1936, **34**, 437–448.)— The present investigation follows previous work inaugurated to determine whether any definite varieties of Shea trees exist in Nigeria and the Gold Coast, and whether there are any differences in yield and quality of the fat obtained from individual trees (*Bull Imp. Inst.*, 1930, **28**, 123; 1931, **29**, 407; 1933, **31**, 334).   Results are now tabulated of the examination of a further series of 21 samples of nuts from the same trees on the Yendi Shea Reserve from which previous samples were taken (*Bull. Imp. Inst.*, 1932, **30**, 282).   The trees were selected to represent as great a range of variation as possible, especially in bark formation and leaf shape, and a description of each of the 21 samples of nuts and kernels is given.   The percentage of moisture in the kernels varied from 6·7 to 13·4, with an average of 7·9 per cent. The kernels as received contained 42·6 to 52·7 per cent. of oil, with an average of 48·7; or 47·1 to 56·9, with an average of 53·1 on the moisture-free kernels.   The oils contained 3·6 to 8·3 per cent. of unsaponifiable matter with an average of 4·9 per cent.   The results, together with those previously obtained, indicate that the oil-content of nuts from a particular tree is liable to vary from year to year, as does also the proportion of contained unsaponifiable matter.   The provisional conclusion drawn from the results of the examination of the previous series of samples, and also from Nigerian samples, namely, that the lower the proportion of oil in the kernel the higher the percentage of contained unsaponifiable matter, receives still further support.   D. G. H.

Some Ray-liver Oils.   M. Tsujimoto.   (*J. Soc. Chem. Ind. Japan*, 1936, **39**, 397B.)—The liver oils of 5 members of the family *Rajidae* (skates) caught off Northern Japan have been examined.   The proportion of oil in the livers varied between 30 and 61 per cent., and the oils were yellow or orange-yellow liquids, giving slight blue colours with antimony trichloride.   The unsaponifiable matter from each oil was a yellow or orange-yellow crystalline mass of comparatively high m.p. and consisting mainly of cholesterol.   The oils tabulated below were

from the following species:—*Raja kenojei* (1*a*) and (1*b*); *R. porosa* (2); *R. smirnovi*, (3); *R. karagea*, (4); and *R. isotrachys*, (5).

Oils				(1a)	(1b)	(2)	(3)	(4)	(5)
Sp.gr. at 15/4° C.	..	..	..	0·9315	0·9359	0·9325	0·9310	0·9270	0·9195
n_D^{20}	..	..	..	1·4839	1·4870	1·4840	1·4825	1·4810	1·4750
Saponification value ..	..	..		184·3	183·3	183·5	181·4	180·9	176·5
Iodine value (Wijs)	..	..		203·5	235·0	203·2	190·1	176·8	130·9
Acid value	..	..	..	0·61	1·56	0·10	1·51	0·69	0·27
Unsaponifiable matter, per cent.		..		1·28	1·57	2·25	2·32	3·63	3·60
Fatty acids									
M.p. ° C.	..	..	..	29–30	32–33	27–28	30–31	27–28	clear at 25–26
Neutralisation value ..	..	..		192·1	190·0	194·1	193·0	195·5	188·3
Iodine value	..	..	..	210·8	240·2	209·1	194·7	185·3	132·6
Ether-insol. bromides, per cent.		..		70·7	89·0	72·0	67·2	55·4	28·2

D. G. H.

Liver Oils of some Deep Sea Fish. M. Tsujimoto and H. Koyanagi. (*J. Soc. Chem. Ind. Japan*, 1936, **39**, 397–398B.)—The four fishes whose liver oils were examined were caught at 400 to 500 fathoms depth off North Japan, and comprised one member of the *Gadidae* family, *Antimora rostrata* Gunther (*a*), and three members of the *Macruridae*, grenadiers or rat tails, *Coelorhynchus productus* Gilbert and Hubbs (*b*), *Macrurus fasciatus* Gunther (*c*), and *M. armatus* Hector (*d*). *Antimora* yielded 52·9 per cent. of liver oil, *Coelorhynchus* 32·9, and *Macrurus armatus* 32·3 per cent. The following values were obtained for the oils:

Liver oils				(a)	(b)	(c)	(d)
Sp.gr. at 15/4° C.	..	..	..	0·9151	0·9266	0·9201	0·9168
n_D^{20}	..	..	..	1·4736	1·4792	1·4784	1·4739
Saponification value ..	..	..		171·8	182·8	173·9	174·0
Iodine value (Wijs)	..	..		112·8	169·3	136·7	116·8
Acid value	..	..	..	0·58	1·09	4·76	0·53
Unsaponifiable matter, per cent.		..		2·68	0·95	4·57	1·31
Fatty acids							
M.p.				liquid at 15° C.	clear at 30° C.	14–15° C.	15° C.
Neutralisation value		..	..	183·9	192·4	185·7	185·6
Iodine value	..	..	..	117·2	175·0	129·9	120·4
Ether-insoluble bromides, per cent.		..	.	17·8	50·6	27·2	19·1

With antimony trichloride the blue colours given by (*a*), (*b*) and (*d*) were far inferior to those obtained with cod-liver oil. The solid constituents of the crystalline orange-yellow unsaponifiable matter in each oil consisted mainly of cholesterol. The fatty acids of (*a*) and (*d*) were converted into the methyl esters and fractionally distilled, and the results showed that the component acids were mostly of the C_{18}, C_{20}, C_{22}, and C_{24} groups. Palmitic acid was present only in small amount, probably accounting for the low m.p. of the fatty acids.   D. G. H.

Colouring of Cheese Crusts. J. Pien. (*Ann. Falsif.*, 1936, **29**, 472–483.)—For cheese of Dutch type practically the only effective colouring matter that can be used in France under the present regulations is alkanet, and the present study aims at showing that in every way Sudan IV would be more suitable. From the point of view of technique it is exceedingly difficult to get extracts of alkanet of similar colouring capacities. Further, since alkanet is red in acid and blue in

alkaline medium, and the cheese at the moment when it is covered by the red paraffin wax is slightly alkaline, it follows that in time there will be a change of colour towards violet. If fermentation occurs under the paraffin layer (and this will happen if the paraffin wax is applied at too low a temperature), this change in colour will begin in a few days. On the other hand, if the temperature of the paraffin wax is too high, an immediate colour-change occurs in the alkanet to dark violet. Sudan IV is not affected by the alkalinity of the crust, the colour remains entirely in the paraffin layer, and no discoloration of the cheese itself occurs. Sudan IV is quite safe to use, for a solution of the dyestuff in butter was fed as the only food to rats for several days, with no ill effects of any sort. Moreover, it is used in other countries for colouring the outside layer of cheese of the Dutch type. D. G. H.

New Differential Colour Reactions of the Cardiotonic Glucosides: Digitoxin, Strophanthin K, Ouabain and German Digitalin. J. A. Sanchez. (*J. Pharm. Chim.*, 1936, **12**, 549–558.)—These cardiotonic glucosides can be detected and distinguished from one another by colour reactions. The following reagents are required:—(*a*) Vanillin in hydrochloric acid; this is prepared by dissolving 0·30 g. of pure vanillin in 100 ml. of conc. hydrochloric acid, free from iron. (*b*) *p*-Dimethylaminobenzaldehyde reagent; four drops of sulphuric acid (sp.gr. 1·83) are added to a solution of 0·10 g. of *p*-dimethylaminobenzaldehyde in 20 ml. of 95 per cent. alcohol. (*c*) Bromo-sulphuric acid reagent; one drop of saturated bromine water is added to 20 ml. of sulphuric acid. *Methods.*—Either the crystalline glucoside or the official 0·1 per cent. pharmaceutical solution is used. With the former, the procedure is as follows:—*Digitoxin*: A fraction of a milligram dissolved in two drops of acetic acid is warmed for three minutes with 10 drops of reagent (*a*), in a test-tube in a boiling water-bath. The reddish colour first formed changes afterwards to an indigo-blue which is permanent. On addition of 1 to 2 ml. of acetic acid the colour is unchanged. *Strophanthin K*: A few milligrams in 2 drops of acetic acid are examined by the method described above. At the end of three minutes a deep blue turbid liquid is obtained; this becomes clear after the addition of acetic acid; the colour is not permanent. *Ouabain*: A fraction of a milligram is evaporated to dryness with 1 ml. of (*b*), in a porcelain dish on a water-bath. A very characteristic violet colour is obtained on acidifying with a few drops of acetic acid. *German digitalin* (digitalinum verum): Quantities of the order of 0·1 mg. are examined. The solid, mixed with 1 ml. of (*b*), is evaporated to dryness on a boiling water-bath. As the solvent evaporates, a series of zones of red colour is observed. The residue is dissolved in 20 drops of acetic acid, and a liquid miscible with water is obtained. This has a deep red, stable colour, like that of eosin.

As the glycerin and alcohol in the official solutions interfere with the colour reactions, they must be eliminated before the tests are applied. The methods of elimination are described. From digitoxin solutions, the solid is obtained for examination, while, from solutions of ouabain and German digitalin, aglucones are obtained as products of hydrolysis. The colour reactions of ouabain and German digitalin are due to the agluconic nuclei in their molecules. The colour reaction of

German digitalin is also given by digitoxin. These compounds may be distinguished from one another by treatment with (c), which gives a cherry-red colour with German digitalin, but no colour with digitoxin. E. B. D.

Alkaloid of *Equisetum palustre*. E. Glet, J. Gutschmidt and P. Glet. (*Hoppe Seyler's Z. phys. Chem.*, 1936, **244**, 229–234.)—An alkaloid and a hydrocarbon have been obtained from *Equisetum palustre*. The hydrocarbon had the formula $C_{21}H_{42}$, and was slightly soluble in chloroform, glacial acetic acid, alcohol and ether, less soluble in ethyl acetate or dilute acetic acid, and almost insoluble in water or dilute mineral acids. The alkaloid, which has been named "palustrin," had the formula $C_{12}H_{24}N_2O_2$. It was very soluble in water, slightly soluble in chloroform and ethyl acetate, less soluble in ether, and only difficultly soluble in benzene and petroleum spirit. The hydrochloride of the alkaloid crystallises in long white needles or rectangular cubes, having m.p. 181° C. The pharmacological action and the chemical constitution of this new alkaloid are being investigated.
S. G. S.

Examination of *Phosphas Natricus Acidus*. M. P. H. Sitsen. (*Pharm. Weekblad*, 1936, **73**, 1575–1576.)—According to the *Codex Med. Nederl.* a solution of 138 mg. of sodium acid phosphate in 10 ml. of water containing dimethyl yellow should require 19·8 to 20·2 ml. of 0·1 N alkali to produce a red colour. According to Kolthoff, however, ("Mass-analyse," Vol. II, p. 139) dimethyl yellow has a pH range of 2·9 to 4·0, and since a solution of the pure salt (sodium acid phosphate?) has a pH value of 4·4, the above method cannot be accurate. If, however, titration is carried out with 0·1 N sodium hydroxide solution, free from carbon dioxide, in the presence of thymolphthalein (pH range, 9·4 to 10·6, *cf.* Kolthoff, *loc. cit.*, p. 140), an accuracy of 0·5 to 1 per cent. is obtainable, and 10 ml. are required to convert the sodium acid phosphate into the diphosphate; a 0·05 M solution of the latter has pH 9·6. J. G.

Biochemical
Selective Adsorption of Enzymes by Cellulose. H. Tauber. (*J. Biol. Chem.*, 1936, **113**, 753–757.)—Cotton exerts a selective adsorption towards enzymes, and therefore the use of this material for the filtration of enzyme solutions should be avoided, since much of the active material is adsorbed. If such solutions require clarifying, a centrifuge should be used, or, alternatively, glass-wool or open-texture, "fast" filter-paper. Adsorption of enzymes by cotton may prove a useful property in selective concentration or in testing the purity of crystalline enzymes, for the enzyme is not contaminated, as it may be when other adsorbents (inorganic gels, tannins, etc.) are used. S. G. S.

Activation of Fat-Catalase by Heat. J. Bodnár and J. Bártfai (Baubach). (*Hoppe Seyler's Z. phys. Chem.*, 1936, **244**, 225–228.)—The decomposing action of the fat from the pig on hydrogen peroxide, and the activity of a cell-free solution of fat-catalase prepared from the same source, are increased considerably (to a maximum of 112 per cent.) by warming for 2 hours. The optimum

temperature for this heat activation is, for summer fat, 31° C., for winter fat, 42° C., and for the solution of the fat-catalase 45° C. The heat activation, at least for the fat-catalase solution, cannot be explained on the basis of haemolysis.

S. G. S.

Studies on Carotenoids IV. The Carotenoid of *Genista tridentata*. K. Schön and B. Mesquita. (*Biochem. J.*, 1936, **30**, 1966–1969.)—The principal carotene of the flower of *G. tridentata* proved to be β-carotene, but a little α-carotene was present. Both of these, together with lutein, have been isolated in the crystalline state, and it is also probable that other carotenoids were present. Since xanthophyll is related, in chemical structure, to α-carotene, whereas the hydrocarbons present in this case belonged to the β-carotene series, it is suggested that lutein may be related *in vivo* to β-carotene and *in vitro* to α-carotene. Sitosterol and another sterol, not identified, were also isolated.

S. G. S.

The Provitamin of Egg Sterols. A. Windaus and O. Stange. (*Hoppe Seyler's Z. phys. Chem.*, 1936, **244**, 218–220.)—Boer *et al.* (*Konink. Akad. Weten. Amsterdam, Proc.*, 1936, **39**) have stated that in a commercial cholesterol, from an unnamed source, they found some ergosterol and suggested adulteration. The authors report that they also have found ergosterol in samples of cholesterol from animal sources, in material which could not have been adulterated. In one experiment, cholesterol from dried Chinese egg-yolk contained 0·18 per cent. of the provitamin, as determined by ultra-violet absorption. A solution of this was adsorbed on a column of aluminium hydroxide and subsequently eluted with a mixture of petroleum spirit, benzene and methyl alcohol in the ratio of 250 : 250 : 1·2. From an original amount of 5 kg. worked up in 35-g. batches, 4·2 g. of cholesterol containing 19·4 per cent. of the provitamin was obtained. This was re-adsorbed on a smaller column and eluted, and a yield of 0·745 g. of cholesterol with a provitamin content of 81·4 per cent. was obtained. Another adsorption and elution gave 0·423 g. containing 100 per cent. of the provitamin. This product, after re-crystallisation from a methyl alcohol–ether mixture had m.p. 155° C. Further re-crystallisation raised the m.p. to that of ergosterol (163° C.). The acetate and the *m*-dinitro-benzoate of the new substance gave no depression when mixed melting-points with the corresponding esters of ergosterol were taken. Catalytic hydrogenation of the provitamin with hydrogen and palladium black gave an α-ergostenol which, when mixed with α-ergostenol from ergosterol caused no depression of the m.p. It is therefore claimed that the provitamin isolated from an animal source, in this case egg-yolk, is identical with ergosterol obtained from the usual vegetable sources. It is suggested that the ergosterol was derived from the food of the hens.

S. G. S.

Colorimetric Estimation of Urinary Oestrin. G. Pincus, G. Wheeler, G. Young and P. Zahl. (*J. Biol. Chem.*, 1936, **116**, 253–266.)—The various methods for colorimetric determination of urinary oestrin have been compared on the oestrone and oestriol fractions of human and rabbit urines. It was found that the phenolsulphonic method of Cohen and Marrian (*Biochem. J.*, 1934, **28**, 1603) gave reliable results for the oestriol content in human pregnancy urines of the

6th to 9th months of pregnancy, but for earlier stages high results were obtained, owing to the presence of inactive materials which gave coloured compounds under the conditions of the test. Oestrone fractions always contained these substances, and results were therefore always high. A rough correction was possible by assuming that the slope of the absorption curve at 580–600$m\mu$ was correlated with the amount of other substances interfering at 514$m\mu$, and making the appropriate allowance. The reacting material in rabbit urines consisted almost completely of physiologically inactive material. The phenolsulphonic acid test of Cartland *et al.* (*J. Biol. Chem.*, 1935, **109**, 213) was found difficult to use with impure extracts because cloudy solutions formed and the colour faded rapidly. The benzoyl chloride test of Görtz (*Biochem. Z.*, 1934, **273**, 396) was found capable of distinguishing oestrone and oestradiol from oestriol, giving a more intense colour with oestrone than with oestriol. With the oestrone fractions of human pregnancy urine this test gave results practically identical with those obtained by the phenolsulphonic acid test, but with rabbit urine extracts the results obtained by this method were double those obtained by the phenolsulphonic acid method. The David test for oestriol (*Acta brev. Neerland.*, 1934, **4**, 464) was found to be specific for the crystalline hormone, but could not be used for extracts because of the formation of cloudy solutions.   S. G. S.

Bacteriological

Use of Micro-Organisms in Sugar Analysis. Quantitative Differentiation of Fructose and Mannose. T. F. Nicholson. (*Biochem. J.*, 1936, **30**, 1804–1806.)—*Gaffkya tetragena* (*Micrococcus tetragenus*) has been used for the separation of fructose and mannose present in dilute solutions. When the use of this organism was combined with that of *Proteus vulgaris* and *Monilia krusei* it was possible to analyse mixtures of glucose, fructose and mannose and to determine added fructose and mannose in blood and urine filtrates. The scheme of analysis for sugar mixtures published by Harding and Nicholson (*Biochem. J.*, 1933, **27**, 1082) has been modified to include the use of *G. tetragena* in the following manner:—

(a) Determine glucose, fructose and mannose by *M. krusei.*

(b) Determine galactose in the residual fluid from (a) by *Sacch. marxianus.*

(c) Determine glucose and fructose by *G. tetragena;* (a)—(c) = mannose.

(d) (In absence of galactose) determine glucose by *P. vulgaris;*
    (c) — (d) = fructose.

(e) (In presence of galactose) determine fructose and mannose in residual fluid from (d) by *M. krusei;*
    (a)—(e) = glucose, (e) — mannose = fructose or (c) — glucose=fructose.

S. G. S.

Agricultural

Determination of Rotenone. R. R. Le G. Worsley. (*J. Soc. Chem. Ind.*, 1936, **55**, 349–357T.)—The method adopted for the determination of rotenone has the same basis as previously described methods, *viz.* extraction with a solvent, formation of a rotenone–carbon tetrachloride complex, and purification of the

complex with alcohol, but several disadvantages have been overcome, and the percentage of rotenone obtained is higher. Percolation is carried out in one of three sizes of tubes—¾ in. diam. for 10 to 20 g. of material, 1 in. for 20 to 40 g., and 1½ in. for 40 to 100 g., each tube about 18 inches long. The three tubes slide inside other tubes, preferably of metal, permanently fixed in the water-jacket. The inner tubes are drawn out at their lower ends and pass through rubber stoppers to filter-flasks immersed in cold water. In the tapering ends of the two larger tubes are placed perforated Gooch crucible discs, on which rest discs of filter-paper with a wad of cotton-wool. By using this apparatus the time of extraction is reduced to between ¾ and 2½ hours, depending on the amount of root used. The most satisfactory solvent was found to be ethyl acetate. The use of decolorising charcoal (5 per cent. for derris and 10 per cent. for Mundelea), mixed with the powdered material before its extraction, results in a slight but definite increase in the purity of the rotenone and carbon tetrachloride complex. The root or Mundelea bark is cut up into small pieces, dried in the air for 1 to 2 weeks, ground in a high-speed disintegrator, and bottled. The samples then contain 3 to 7 per cent. of moisture, and the amount taken for extraction should yield about 1 g. of rotenone. After extraction a weighed amount of pure rotenone is added to the resins to bring the content to at least 40 per cent., and 1 g. is added even if the amount is already up to, or above 40 per cent. This is stirred into the heated resins, and the mixture is warmed until solution takes place and then left to crystallise overnight in a desiccator containing carbon tetrachloride. The crystals are broken up, filtered off, washed with solvent (carbon tetrachloride + rotenone) until no further colour is obtained, and dried for 6 hours at about 40° C., and the weight, multiplied by 0·719, gives the amount of crude rotenone. The purity of the crude carbon tetrachloride complex is, except for occasional samples, between 91 and 96 per cent., and the mean value, 94 per cent., may be assumed in approximate routine work. Determination of the purity of the complex by optical rotation gives results, on the average, 2·6 per cent. too high. The purity can be accurately determined by recovering the rotenone from the complex by triturating with absolute alcohol (5 ml. per g.) saturated with rotenone, leaving the mixture overnight in a desiccator containing some alcohol, and filtering off, washing with alcohol and drying at 100° C. The purity of the recovered rotenone is about 99·2 per cent. and can be checked by optical rotation in benzene solution. Six different samples of *Derris elliptica*, two each of *D. malaccensis*, one of *D. polyantha*, and two of *Mundelea suberosa* bark were examined in detail, and full results are tabulated for three samples.   D. G. H.

Determination of Fumigants. VII. Determination of Sulphur Dioxide and Sulphur Trioxide from Burning Sulphur. O. F. Lubatti. (*J. Soc. Chem Ind.*, 1936, **55**, 344T–346T.)

—The paper describes a macro method for the determination of sulphur dioxide and sulphur trioxide and a micro method for the determination of sulphur dioxide in sulphur dioxide fumigant obtained by burning sulphur; the gas sample is collected in the evacuated flasks of Page's apparatus (*J. Soc. Chem. Ind.*, 1932, **51**, 369T; 1935, **54**, 421T), 1-litre flasks being used for the macro method and 20-ml. flasks for the micro method. The gases are absorbed

in sodium hydroxide solution, glycerin being used as a stabiliser for the sodium sulphite formed. For the macro determination of sulphur dioxide the indirect iodimetric method of Kolthoff ("Volumetric Analysis," Vol. II, p. 399) is accurate but inconvenient. Two satisfactory methods are: (a) a modification of that due to Vincent and Richards (*J. Pharm. Chim.*, 1900, **10**, 482; 1902, **16**, 207), in which iodine is liberated in the solution containing sodium sulphite by the interaction of potassium iodide and potassium iodate after acidification, and (b) direct titration with iodine. For the first method the sampling-flask contains 50 ml. of 0·1 N sodium hydroxide solution with 5 per cent. of glycerin, and not more than 80 mg. of sulphur dioxide are absorbed. The liquid is neutralised with 0·1 N sulphuric acid to the end-point of methyl orange. Ten ml. of a 10 per cent. solution of potassium iodide are added, followed by 50 ml. of 0·05 N potassium iodate solution and 50 ml. of 0·2 N sulphuric acid. The iodine liberated is titrated with 0·05 N sodium thiosulphate solution, starch solution being used as an indicator. A blank test is made, and the quantity of sulphur dioxide is calculated by difference. For the concentrations involved there is no significant difference between the results given by the indirect iodimetric method, the potassium iodate method and direct titration. As iodine volatilises from aqueous solutions, even in the presence of potassium iodide, it is advisable to use an all-glass titration system, such as that described by the author (*J. Soc. Chem. Ind.*, 1935, **54**, 424T).

For the determination of sulphur dioxide and sulphur trioxide the sampling-flask is charged with 50 ml. of 0·1 N sodium hydroxide solution containing 5 per cent. of glycerin. After the gas sample has been collected the excess of alkali is titrated with 0·1 N hydrochloric acid to the end-point of methyl orange, a colour standard being used for comparison. The solution is diluted with 200 ml. of distilled water containing 5 per cent. of glycerin, and 30 ml. of 0·2 per cent. starch solution is added. The sulphite is titrated with 0·05 N iodine solution, delivered from a burette incorporated in an all-glass system. A positive correction of 1 per cent. should be introduced when the concentration of sulphur dioxide in the solution exceeds 0·04 per cent. by weight.

For the micro-determination of sulphur dioxide the sampling flask is charged with 2 ml. of 0·05 N sodium hydroxide solution containing 5 per cent. of glycerol. After the gas sample has been collected the excess of alkali is neutralised with 0·05 N hydrochloric acid, methyl orange being used as indicator; 0·5 ml. of 0·2 per cent. starch solution is added, and the sulphite is titrated with 0·01 N iodine solution delivered from a 5-ml. burette, graduated in 0·02 ml., incorporated in an all-glass system. The above-mentioned correction of 1 per cent. should be introduced. The quantity of sulphur trioxide absorbed is too small to be determined volumetrically. E. M. P.

Determination of Lactose in Mixed Feed. D. A. Magraw, L. E. Copeland and C. W. Sievert. (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 605–607.)—The method is a modification of that of Magraw and Sievert (*Ind. Eng. Chem. Anal. Ed.*, 1935, **7**, 106) and gives more accurate results. It involves the conversion of starch and sugars by means of animal diastase, thereby reducing the time required for subsequent fermentation. The sample (16·25 g. weighed to within 0·03 g.) is mixed in

a 300-ml. flask with about 200 ml. of distilled water and digested on the water-bath for 30 minutes. When cold, the mixture is made up to 300 ml. and centrifuged. Of the supernatant liquid, 150 ml. are treated in a 200-ml. flask with 0·25 g. of animal diastase at 52 to 55° C. for 25 to 30 minutes, after which the flask is placed in boiling water for 15 minutes. When the flask has cooled, a further 0·25 g. of animal diastase is added, and the conversion and subsequent heating are repeated. The mixture is cooled to room temperature and, after the addition of 75 mg. of invertase-melibiase scales and 1·5 to 2 g. of bakers' yeast, the flask is plugged with sterile cotton-wool and fermentation is allowed to proceed at 26·5 to 30° C., with a permissible upper limit of 33° C., for 17 to 18 hours. The liquid is then cooled, made up to 200 ml., and centrifuged. Of the supernatant liquid, 190 ml. are concentrated by boiling to 25 or 50 ml., washed into a 100-ml. flask with hot water, treated with 10 ml. of saturated lead acetate solution, diluted to 100 ml. and centrifuged. To 50 ml. of the clear solution in a 100-ml. flask 2·5 ml. of a 5 per cent. solution of mercuric chloride are added, and the mixture is allowed to stand for 15 minutes with repeated shaking. After the addition of 5 ml. of 20 per cent. phosphotungstic acid solution, the liquid is made up to 100 ml. and filtered if necessary. Hydrogen sulphide is passed in, the precipitate is filtered off, and 50 ml. of the filtrate are boiled to remove hydrogen sulphide, the water lost being replaced. Lactose is then determined by the Munson and Walker method (*Assoc. Off. Agric. Chem., Methods of Analysis*, 1930 Edition, p. 379), a Jena fritted glass crucible (1G4) being recommended for filtration. After being thoroughly washed the precipitate of cuprous oxide is dissolved in 5 ml. of hot dilute nitric acid (1 + 1), 0·5 g. of urea is added (Lyle, *Ind. Eng. Chem. Anal. Ed.*, 1936, **8**, 200), and the solution is boiled for 2 minutes. Copper is determined by titration with standard sodium thiosulphate solution (19 g. of the crystallised salt per litre), and the corresponding amount of lactose is obtained from Walker and Munson's table (*loc. cit.* p. 514). The number of grams of material used in the final aliquot portion is given by

$$\frac{150}{(300-8)} \times \frac{190}{200} \times \frac{50}{100} \times \frac{50}{(100-1)} \times 16{\cdot}25 = 2{\cdot}00 \text{ grams}$$

a total correction of 9 ml. having been made for volumes of precipitates. The percentage of lactose in the original material is given by $\frac{(x - 0{\cdot}006)100}{0{\cdot}96 \times 2}$, where $x =$ lactose determined, 0·006 is a correction for a blank determination, and 0·96 is a correction for loss of lactose during fermentation. Examples of determinations of known amounts of lactose added to feeding stuffs are given, and these show that the method gives accurate and consistent results.   A. O. J.

Water Analysis

Photometric Determination of Silicate in Sea Water. R. J. Robinson and H. J. Spoor. (*Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 455–457.)—The colorimetric method of Dienert and Wandenbulcke (*Compt. rend.*, 1923, **176**, 1478), various modifications of which have been proposed by different workers, has been investigated. The method, which involves colorimetric determination of the

yellow silico-molybdate complex formed in the acidified silicate solution on the addition of ammonium molybdate, was studied by measuring the transmission of light through the coloured solution with the aid of a Zeiss Pulfrich photometer. It was found that the colour developed to full intensity within 3 minutes, and did not fade until at least 2 hours later. Temperature had no effect on the intensity of colour between 6° and 22° C. A repetition of the work of Swank and Mellon (*Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 348) with fresh waters confirmed their findings that the Winkler reagent with hydrochloric acid (*Z. anorg. Chem.*, 1914, **27**, 511) yielded 1·09 times as much colour for the same amount of silicate as the reagent of Dienert and Wandenbulcke with sulphuric acid. In synthetic sea water, the colour-intensity was essentially the same with either reagent. The maximum development of colour occurred between pH values of 1·5 and 2·3; with increased acidity, slightly less colour developed. Tests were made of the applicability of permanent colour standards of potassium chromate and sodium borate solution (Swank and Mellon) and picric acid (King and Lucas, *J. Amer. Chem. Soc.*, 1928, **50**, 2395). The chromate standard was found for various reasons to be preferable. The applicability of Beer's law relating to variation in colour with dilution was studied by plotting the negative logarithm of the light transmission against silicate concentration. The law was found only to apply approximately with concentrations of silicate greater than 0·20 mg.-atomic proportions of silicon per litre, but it held satisfactorily at lower concentrations. For the same silicate concentration, less silicomolybdate colour developed in a sea-water medium than in fresh water, the ratio being 1 : 1·16. A correction factor according with this ratio is therefore required if permanent colour standards are used. S. G. C.

Organic

Study of the Resorcinol Reaction of Oxalic Acid. M. Pesez. (*Bull. Soc. Chim.*, 1936, [5], **3**, 2072–2074.)—The colour reaction of oxalic acid with resorcinol previously described (*Bull. Soc. Chim.*, 1936, [5], **3**, 676) has been modified as follows:—One ml. of cold sulphuric acid (1 : 2) is added to 5 ml. of the solution to be examined, followed by a few pieces of zinc-copper couple or 2 drops of a 10 per cent. aqueous solution of copper sulphate and about 1 g. of zinc. After about 3 minutes, 0·2 ml. of the reduced solution, which contains glyoxylic acid, is added to 2 ml. of conc. sulphuric acid (sp.gr. 1·84) in a test-tube, followed by 0·1 ml. of Denigès' resorcinol reagent (2 per cent. aqueous solution of resorcinol). A pale blue colour develops in the cold and becomes intensely blue on heating on a water-bath. The reaction is very sensitive and will detect 0·10 g. of oxalic acid in 1 litre of distilled water, or 0·02 mg. in the sample under examination; it can be carried out in the presence of hypochlorous, chloric, bromic, nitrous, nitric, and chromic ions. If hydrobromic or hydriodic ions are present, the procedure is modified as follows:—Two ml. of sulphuric acid are placed in a test-tube and mixed with 2 drops of the resorcinol reagent; 4 drops of the reduced solution are run down the side of the tube. In the presence of oxalic acid a blue ring forms at the junction between the liquids; in presence of tartaric acid and absence of oxalic acid a violet-rose ring forms.

This reaction invalidates the colour test for tartaric acid if oxalic acid is present. The following procedure is recommended for the elimination of the oxalic acid. Five ml. of the solution are treated with 3 to 4 drops of dilute sulphuric acid (1 : 2), followed by the gradual addition of calcium carbonate, with shaking, a slight excess of calcium carbonate being added. Glacial acetic acid is then added, drop by drop, until the odour persists (3 to 4 drops after the cessation of effervescence), and the liquid is treated with a few crystals of a neutral soluble sulphate (sodium, potassium or magnesium sulphate). After shaking, the liquid is filtered through a pleated paper, and the filtrate is treated with 1 ml. of sulphuric acid (1 : 2), 2 drops of a copper sulphate solution and a piece of zinc. After 2 to 3 minutes the reduction product is condensed on a water-bath with the resorcinol or bromoresorcinol reagent in conc. sulphuric acid, using 2 drops of solution and 2 drops of reagent in 2 ml. of acid. The rose-violet colour of the Molher-Denigès reaction or the blue colour of the author's reaction is then obtained in the presence of tartrates.   E. M. P.

Determination of Semicarbazones. S. Veibel. (*J. Pharm. Chim.*, 1936, **24**, 499–502.)—The author's method (*Bull. Soc. Chim.*, 1927, [4,] **41–42**, 1410; Abst., ANALYST, 1928, **53**, 53) for the determination of semicarbazones is as follows:—A weighed amount of semicarbazone is boiled with sulphuric acid (10 to 30 per cent.) for $\frac{1}{2}$ to 1 hour; the semicarbazone is hydrolysed with formation of aldehyde (or ketone), hydrazine, and ammonia. The hydrazine is removed by oxidation with iodic acid, the iodine formed is distilled off until the colour of the iodine has disappeared, and the ammonia is then determined in alkaline solution by the Kjeldahl process. This method is compared with that of Harlay (cf. *J. Pharm. Chim.*, 1936, **8**, 23, 199), which is based on the determination of hydrazine. The latter process is not satisfactory for the determination of semicarbazones of formaldehyde, cinnamic aldehyde and citral, nor for those of α-ketonic acids, owing to the formation of aldazines. Veibel's method is not satisfactory for the semicarbazone of formaldehyde, but is satisfactory for those of cinnamic aldehyde, of citral, and of pyruvic acid. The derivative from citral was a mixture of the α- and β-compounds; and results indicate that both α-citral and β-citral can be determined by this method. The following analytical results were obtained:

| | | | Molecular weights | |
Semicarbazone of			Found	Calculated
Cinnamic aldehyde	..	..	190·7	189·1
Citral (α- and β-)	..	..	210·1	209·2
Pyruvic acid ..	..	..	(a) 145·3	145·1
			(b) 144·9	

[The m.p. for the semicarbazone of pyruvic acid given by Beilstein is about 200° C. Veibel, with this compound re-crystallised, finds 220–222° C. by warming slowly and 246–248° C. with the Maquenne block.]   E. B. D.

Relationship between Chemical Composition and Mechanical Strength in the Wood of English Ash (*Fraxinus excelsior*, Linn.). K. R. Bamford and E. D. van Rest. (*Biochem. J.*, 1936, **30**, 1849–1854.)—The percentage amount of Cross and Bevan cellulose increased in passing from the periphery

towards the pith of ash wood. The percentage amounts of lignin and extractives tended to decrease in the same direction, while the furfural-yielding complexes remained virtually constant in amount throughout a cross-sectional disc of any one tree. It was also found that the Cross and Bevan cellulose of ash heartwood contained more hexosans and less furfural-yielding material than the corresponding component of the sapwood. The defect known commercially as "black-heart" was associated not with any irregularities in the proportions of the three major components present in the wood, but apparently with some minor constituent. Variations in the Cross and Bevan cellulose appeared to be the cause for the variations in crushing strength in pounds per square inch of wood substance. In the material examined, the specific gravity, the cellulose-content and the strength increased in a direction from the periphery towards the pith.   S. G. S.

Scientific Examination of Handwritings in Country-made Carbon Inks. S. Dutt. (*Document Investigation*, 1936, **4**, 3–6.)—The inks in question are used largely in India in rural districts, and, to a less extent, in towns. They are prepared by suspending the soot collected from smoky lamps or burning oil in a gum made from glue, gum arabic, or gum acacia, or from starch- or sugar-products, and as they are not preserved they decompose in time, becoming more black at first, although they become fainter with age, and may eventually be obliterated. This is due to the fact that the pigment does not penetrate below the surface of the paper to the extent of more than 10 per cent. of its thickness, and, although the gum may penetrate to the extent of 50 per cent. of the thickness, it is completely destroyed within 12 years by oxidation, especially in the presence of air and moisture. This destruction of the binding medium facilitates the removal of the carbon particles by handling, friction, or even contact with another sheet, and it may be demonstrated by the absence of any colour-reaction when a cross-section of the sheet is stained with a solution of phloroglucinol or α-naphthylamine in hydrochloric acid, or by differential staining with Herzberg's stain, osmic acid, safranine or Capri blue. If glue has been used, however, the application of a 10 per cent. solution of phosphomolybdic acid to the surface of the paper will reveal the original writing as a light green-brown stain. Lampblack from kerosene oil has the appearance under the microscope of thick, coarse, brown-black granular masses, and in plane-polarised light the tips of the granules glisten against a dark background. Turpentine oil or rosin yields a similar pigment which has a finer structure, whilst pigments from non-drying oils (*e.g.* castor oil, mahua oil and coconut oil) are still finer and have a glossy violet-black shade. Linseed, tung, groundnut, sesame or mustard oil and fish oils yield the most uniform and most permanent pigments, and these vary in colour from deep blue to jet black. Most of these pigments contain from 3 to 10 per cent. of the original oil, as well as characteristic resinous compounds, and in filtered ultra-violet light these fluoresce as follows:— Kerosene, bright violet or purple; turpentine, moderately intense green-brown; coconut, castor or mahua oil, dull orange-brown; sesame or groundnut oil, pale brown-yellow; linseed or tung oil, very faint green-yellow; fish oil, light purple-yellow. The fluorescence is rendered more easily visible if the paper is treated with a solvent (*e.g.* ether) and carefully dried, as this spreads the oil, but the

fluorescence decreases in the course of time and disappears after 3 to 5 years if the inks were derived from kerosene, rosin or turpentine, 4 to 6 years for castor, mahua or coconut oil, and 6 to 8 years for the other oils. If fresh handwriting is placed in contact with a fast photographic plate (*e.g.* Ilford "Golden Isozenith") under pressure for 2 weeks in the dark and the plate is then developed in a slow-action, fine-grain metol developer and intensified if necessary, a positive image of the writing results. This is attributed to gases occluded by lampblack when it is formed or evolved as it oxidises, and this property of the ink decreases gradually in the course of time, disappearing in 3 years. This type of writing may be preserved and protected from forgery by immersing the document in a 2·5 per cent. solution of cellulose acetate in acetone for 10 minutes; the excess of liquid is drained off and the document is dried in the shade at room-temperature. The percentage composition of the 22·3 per cent. of solid matter contained in a typical ink was as follows:—Carbon, 77·9; gum, 6·8; oil or fat, 7·3; resinoids, 3·9; ash, 6·3 (contained large quantities of potassium carbonate and sodium chloride, with calcium, magnesium, aluminium, silicon, sulphate and phosphate, and traces of other metals).   J. G.

Inorganic

Hexanitrato Ammonium Cerate as a Standard in Oxidimetry. G. F. Smith, V. R. Sullivan and G. Frank. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 449–450.)—Hexanitrato ammonium cerate $(NH_4)_2Ce(NO_3)_6$, is proposed as a standard material for preparing ceric salt solution for volumetric titration purposes. It is an anhydrous non-hygroscopic salt and may be readily prepared in a pure condition from thorium-free cerium oxide containing praseodymium, neodymium and lauthanum. The salt is easily soluble in dilute sulphuric acid, yielding a solution which is stable under ordinary conditions of storage and also on heating at 100° C. The nitrate ion in the salt was found to have no influence upon the accuracy in the determination of ferrous iron, and was not expected by the authors to interfere with various other determinations for which a ceric sulphate solution is normally employed. It is stated that standard solutions free from nitrate may be prepared, if necessary, by dissolving a known weight of hexanitrato ammonium cerate in sulphuric acid, removing nitric acid by heating, and dissolving the residue in water. The salt is available commercially.   S. G. C.

Volumetric Determination of Mercury. N. H. Furman and H. M. State. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 467–468.)—The method of Spacu and Dick (*Z. anal. Chem.*, 1929, 76, 273), in which mercury is precipitated as mercuric pyridine dichromate, has been modified, the dichromate ion in the precipitate being determined volumetrically. To the mercuric chloride solution (140 ml.) 10 ml. of ammonium dichromate solution (20 per cent.) are added. The liquid is stirred vigorously while 1 ml. of pyridine is added. After standing for 10 minutes, the yellow precipitate is filtered off on a sintered glass filter, a solution containing 0·5 g. of ammonium dichromate and 0·5 ml. of pyridine per litre being used to transfer the precipitate to the filter. The precipitate is washed 6 to 8 times with acetone; air is finally drawn through the filter to cause evaporation of the acetone,

and the filter is placed in a vacuum desiccator for 10 to 15 minutes. The precipitate is then dissolved in dilute acid, and the dichromate ion is titrated either iodimetrically or with ferrous sulphate solution. In test experiments 50 ml. of 1·2 N hydrochloric acid was used to dissolve the precipitate; the ferrous sulphate titration was carried out either potentiometrically or with diphenylamine as indicator. Results very close to the theoretical were obtained with 0·1443 to 0·2654 g. of mercuric chloride. S. G. C.

Analysis of Magnesium Alloys. W. H. Withey. (*J. Soc. Chem. Ind.*, 1936, **55**, 357–359T.)—*Calcium.*—The preferred method for magnesium-calcium alloys is as follows:—A 1 to 2-gram sample is dissolved in hydrochloric acid, and twice the amount of N sulphuric acid required to combine with the calcium is added. The solution is evaporated to dryness on a steam-bath; 50 to 70 ml. of industrial alcohol are added, the mixture is well stirred, and the calcium sulphate, which remains undissolved, is filtered off and washed with alcohol; the calcium is finally determined as oxide after one or more precipitations as oxalate. It was found in tests that, although the theoretical amount of sulphuric acid is enough to fix the calcium, twice the amount is advisable; a greater excess produces more magnesium sulphate than can be dissolved in the alcohol used. The method of precipitation of the calcium from the solution of the alloy by the addition of sufficient oxalic acid to combine with all the calcium and magnesium (precipitation at room temperature), followed by re-precipitation of the calcium oxalate to purify it, was found to lead to slight loss of calcium, to the extent indicated by the following results:

Oxalate method	Sulphate method
3·98 per cent.	4·03 per cent.
0·79 ,,	0·83 ,,
20·56 ,,	20·60 ,,

Cerium and other constituents.—A 1 to 1·5-gram sample of the alloy is dissolved in hydrochloric acid, and N sulphuric acid is added in amount equivalent to twice the amount of metals, other than magnesium, which are present. The solution is evaporated in a shallow wide-mouthed beaker on a steam-bath until all free hydrochloric acid is expelled. The residue is treated with 50 to 70 ml. of industrial alcohol, and the insoluble sulphates are filtered off after settling, and washed with alcohol. After gentle ignition, the precipitate is dissolved in 5 ml. of hydrochloric acid, a few drops of hydrogen peroxide are added, the solution is neutralised with ammonia, and 3 per cent. by volume of conc. hydrochloric acid is added. The rare earths are precipitated by addition of enough oxalic acid to provide an excess of 3 g. per 100 ml.; vigorous stirring of the solution, and allowing it to remain for several hours at 70° to 80° C. (then overnight at the ordinary temperature) are advised. The precipitate of oxalates is filtered off, washed with "dilute acid" and ignited to oxides. The filtrate, containing the calcium, is neutralised with ammonia to precipitate calcium oxalate, and the calcium is determined in the usual manner. The alcoholic filtrate from the insoluble sulphates is evaporated to dryness and the residue is treated with hydrochloric acid and potassium chlorate to destroy traces of organic matter. The residue is dissolved and ammonium

chloride is added.   The solution is neutralised, made just acid with acetic acid, 5 g. of ammonium acetate are added, and the cobalt is precipitated as sulphide at 80° to 90° C.   The cobalt sulphide is filtered off, washed with a slightly acid solution of ammonium acetate saturated with hydrogen sulphide, and the precipitate is converted into cobalt sulphate for gravimetric determination.   The filtrate is made alkaline and saturated with hydrogen sulphide to precipitate the manganese as sulphide; the precipitate is filtered off, washed, and dissolved in 1 : 5 hydrochloric acid saturated with hydrogen sulphide in order to recover traces of cobalt which are sometimes found at this stage.   In the absence of iron and aluminium, the manganese may be determined by means of bismuthate or as sulphate.   In the presence of iron or aluminium it is more convenient to precipitate these two with ammonia before precipitating cobalt with hydrogen sulphide.   A separate determination of manganese may be made by means of potassium persulphate in dilute acid, but the complete elimination of cobalt is very difficult, and the method suggested has proved more satisfactory.   Good results were obtained in test experiments with synthetic mixtures made to simulate magnesium alloys containing a few units per cent. of cerium, calcium, cobalt, manganese and aluminium.

S. G. C.

Detection and Determination of Minute Quantities of Zinc by Means of Diphenylthiocarbazone.   H. Fischer and G. Leopoldi. (*Z. anal. Chem.*, 1936, **107**, 241–269.)—The green solution of the reagent in carbon tetrachloride, chloroform, or carbon disulphide, when shaken with an approximately neutral aqueous zinc solution, turns purple-red.   All other metals, with the exception of cobalt, palladium, and bivalent tin, can be masked by means of thiosulphate. By oxidation of tin to the stannic state and the agency of cyanide, the interference of these three metals is likewise overcome; hence the zinc test is specific for that metal.   The limit of sensitiveness is normally 1 to 2γ in 0·1 to 0·5 ml. of solution. For the lengthy and complicated working directions, reference should be made to the original paper.   W. R. S.

Determination of Lanthanum by means of 8-Hydroxyquinoline. T. I. Pirtea. (*Z. anal. Chem.*, 1936, **107**, 191–193.)—The nitrate solution (50 ml.) is heated nearly to boiling, and treated with 5 ml. of 2 N acetic acid and an excess of alcoholic 3 per cent. solution of the precipitant, followed by 10 per cent. ammonia, drop by drop, until its smell becomes pronounced.   The yellow lanthanum precipitate is allowed to settle for an hour, collected on a porous glass crucible, washed with warm water, and dried at 130° C. to constant weight (La factor, 0·2433).   Alternatively the precipitate may be dissolved in 2 N hydrochloric acid and titrated bromometrically (ANALYST, 1927, **52**, 431); 1 ml. of 0·1 N thiosulphate is equivalent to 0·001167 g. La. (*Sic* in original).   W. R. S.

Rapid Method for the Determination of Selenium in Sulphur.   W. C. Hughes and H. N. Wilson. (*J. Soc. Chem. Ind.*, 1936, **55**, 359–360T.)—The method consists in oxidising the sulphur with nitric acid, removing the nitric acid by evaporation, and ultimately reducing the selenious acid to elemental selenium by means of potassium iodide in the presence of a protective colloid, yielding a

coloured solution which can be matched with standards. A 5-gram sample of the finely-powdered sulphur (150-mesh) is added, a little at a time, to 100 ml. of 94 per cent. nitric acid (sp.gr. 1·50). The solution is evaporated until fumes of sulphur trioxide are given off. [In some samples asphaltic matter is present which offers difficulty in oxidation, and repeated evaporation with nitric acid may be necessary. If the solution still remains coloured on dilution, it is best to match the slight colour by adding caramel solution to the standards before reduction of the selenious acid.] The residue is diluted to 80–85 ml. with water, and the solution is filtered, if necessary, and cooled; 10 ml. of 10 per cent. potassium iodide solution and 1 ml. of 5 per cent. gum arabic solution are added, and the solution is heated at 55° to 60° C. for 15 minutes, and then cooled. One ml. of starch solution (0·2 per cent.) is added, and the blue colour due to the liberated iodine is discharged by the addition of a slight excess of sulphur dioxide solution. The colour of the solution is matched colorimetrically with standards as noted below. Standard selenium solution is prepared by dissolving 0·050 g. of selenium in a few ml. of nitric acid; the solution is evaporated to dryness on a water-bath, and the residue is dissolved in 1 to 2 ml. of nitric acid and diluted to 1 litre. Suitable volumes of this solution are diluted to 80–85 ml., 8 ml. of pure conc. sulphuric acid (equivalent to 5 g. of sulphur) are added, and the liquids are treated in the same way as the test solution, as described above. The limit of sensitiveness of the method is 0·02 mg. of selenium. Samples of seleniferous sulphur which were tested were found to contain 0·01 to 0·02 per cent. of selenium. Sicilian and Texan sulphur are free from selenium. S. G. C.

Colorimetric Determination of Small Quantities of Bromine in the Presence of a large Excess of Chlorine. P. Balatre. (*J. Pharm. Chim.*, 1936, **29**, 409–413.)—The methods of Denigès and Chelle (*Comptes rend.*, 1912, **155**, 721, 1010; Abst., ANALYST, 1912, **37**, 58; *Bull. Soc. Pharm. de Bordeaux*, 1917, **55**, 75) and of Swarts (*Bull. Acad. Belg.*, 1889, **17**, 359), modified by Hahn (*Compt. rend.*, 1933, **197**, 245; Abst., ANALYST, 1933, **58**, 567), proved unsuitable for the determination of bromine in blood. The method of Stenger and Kolthoff (*J. Amer. Chem. Soc.*, 1935, **57**, 831), depending on the conversion of phenol red into bromophenol blue was adopted. The reagents required are: (*a*) phenol red solution, containing phenol red (phenol-sulphonephthalein) 33 mg., $N/10$ sodium hydroxide solution 3 ml., and distilled water to 100 ml.; (*b*) buffer solution, containing N sodium hydroxide solution 500 ml., and N acetic acid, about 650 ml.; (*c*) indicator buffer solution, containing phenol red solution 25 ml., and buffer solution to 500 ml.; (*d*) $N/200$ chloramine solution (0·07 per cent. solution of the sodium salt of monochlorotoluene-*p*-sulphonamide); as this solution does not keep well, its titre should be checked by titration with iodine, and it should be renewed frequently; and (*e*) $N/10$ sodium thiosulphate solution. The method is as follows:— Into a 30-ml. beaker are measured 5 ml. of bromide solution (containing 0·005 to 0·01 mg. of bromine, or 0·0075 to 0·0149 mg. of potassium bromide) and 2 ml. of indicator buffer solution. Two ml. of $N/200$ chloramine solution are measured into a small tube, and poured in one lot into the beaker. The liquid is agitated, and, after 15 seconds, the reaction is stopped by the addition of 3 to 5 drops of

thiosulphate solution. The liquid is poured into a 10-ml. graduated tube, the beaker is rinsed with a few drops of distilled water, and the liquid is made up to 10 ml. The colour is measured with a Pulfrich photometer, using a spectral filter S.57 (wave-length about $572m\mu$). With a depth of 20 mm. the coefficient of extinction is 0·31 for 0·01 mg. of bromine (an absorption of 51 per cent. of the incident light). The intensity of the colour follows the Lambert-Beer law. Under such conditions the error is less than 5 per cent. If a Pulfrich photometer is not available, the colour can be compared with a range of standards.   E. M. P.

Determination of Acetylene in Liquid Oxygen. L. M. Jolson, J. J. Strishevsky and A. B. Bergelson. (*Zavodskaya Laboratorya*, 1936, No. 8, 852–3.)—Five measured litres of liquid oxygen are gradually poured into a vessel containing a copper spiral tube through which compressed air is being blown. When the volume has been reduced to about 100 ml. the gas is transferred to a heat-insulated copper container from which the vaporised oxygen is blown through special wash-bottles each containing 25 g. of freshly precipitated mercuric iodide, 30 g. of potassium iodide, 100 ml. of water, and a fragment of alkali. The precipitate is filtered off, washed with distilled water until free from iodine, transferred to a 250-ml. conical flask and dissolved in boiling nitric acid (1 : 3). The solution is titrated with $N/100$ ammonium thiocyanate solution—

$$HgC_2 + 2HNO_3 = Hg(NO_3)_2 + H_2C_2$$

$$Hg(NO_3)_2 + 2NH_4CNS = Hg(CNS)_2 + 2NH_4NO_3$$
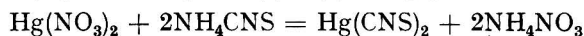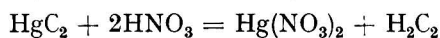
One ml. of $N/100$ ammonium thiocyanate corresponds to 0·00013 g. or 0·11023 ml. of acetylene. The analysis takes 40 to 45 minutes, and the results are accurate to 0·01 ml. of acetylene.   S. P. S.

Microchemical

Microchemical Identification of Ephedrine and Ephetonine. G. A. W. J. O. E. Paris. (*Pharm. Weekblad*, 1936, 73, 1526–1527.)—To the edge of a drop of the solution to be tested is added a fragment of solid potassium oxalate. The hydrochloride of natural ephedrine immediately deposits bundles of needles and prisms which usually form fan-shaped groups, whilst well-formed thin diamond-shaped crystals are produced rapidly by ephetonine (the hydrochloride of the racemic synthetic product). Photomicrographs are provided. Addition of a drop of solution of sodium vanadate to a drop of the test solution produces a fine micro-crystalline precipitate (similar in appearance to calcium oxalate in *Rheum*), and quill-shaped crystals, which are sometimes deformed and frequently grouped, respectively. This latter reaction is the more difficult to carry out successfully.
                          J. G.

Micro and Semi-micro Determination of Sodium and Potassium. M. Hegedüs. (*Z. anal. Chem.*, 1936, **107**, 166–175.)—The macro method of Szebellédy and Schick (ANALYST, 1934, **59**, 502) has been modified for application on a smaller scale. The solution of the mixed alkali chlorides or sulphates* is

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* Szebellédy and Schick found that 0·25 g. of alkali sulphates could be completely converted into iodides by a single evaporation with 2 ml. of pure hydriodic acid, the sulphuric acid being eliminated by reduction, thus, $H_2SO_4 + 2HI = SO_2 + I_2 + 2H_2O$.

evaporated to dryness with 2 ml. of hydriodic acid in a small round-bottomed silica dish. If the dry residue is slightly yellow (free iodine), it is moistened with alcohol and again dried by evaporation. The mixed iodides are heated for 20 to 60 minutes (according to quantity) at 120° to 130° C., and are kept over strong sulphuric acid until ready for separation. The solvent used is a mixture of *iso*butyl alcohol and ether (1 : 1·5) saturated with potassium iodide by 10 minutes' agitation with the finely-powdered pure salt. The prepared solvent, which contains 0·66 mg. of KI (equivalent to 0·19 mg. K_2O) per 10 ml., is stored in the dark in a coloured glass bottle.

If the mixed alkali iodides weigh a few cg., they are extracted three times with 10 ml. portions of solvent, with intervening solution of the residue in hot water and evaporation to dryness. Each 10 ml. of solvent is applied in 2-ml. portions, the salt being thoroughly ground, after each addition, by means of a small glass pestle, for $1\frac{1}{2}$ minutes. When a 10-ml. portion has been added, the solution is left to settle for about two minutes, and decanted from the residue through a filter-paper. The contact between dish and pestle should not be interrupted while the liquid is being poured on the filter, and the lip of the dish must be wiped with a small piece of filter-paper, which is thrown on the filter.

On a micro scale, the extraction is conducted with three 3-ml. portions, each of which is added in portions of 1 ml. with an intervening trituration for $1\frac{1}{2}$ minutes. Ether (2 ml.) is added to the extract prior to filtration with decantation.
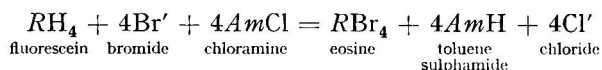
The filtrate, containing the sodium, is received in a 50-ml. glass dish. A few drops of 0·01 N thiosulphate solution and a little ethyl alcohol are added to prevent separation of iodine, due to a possible peroxide-content of the ether. The filter containing the insoluble potassium iodide is washed once with ethyl alcohol, then thoroughly with hot water, the washings being returned to the dish with a drop of hydriodic acid, and evaporated to dryness on the water-bath. The dry residue is ready for another extraction.

For the determination of potassium and of sodium, the insoluble fraction is dissolved in hot water, and the solution is evaporated on a water-bath; the combined extracts also are evaporated to dryness, as described. The iodine in both fractions is determined as follows:—The aqueous solution of the salt is treated with chlorine water until colourless, the iodide being oxidised to iodate. The solution is boiled in a small flask with a little pumice for the expulsion of free chlorine. The cooled solution is treated with 1 ml. of N hydrochloric acid and an excess of potassium iodide, and the liberated iodine is titrated with 0·1 (or 0·01) N thiosulphate solution. The potassium result requires no correction, but a deduction must be made from the sodium value found, for Na_2O equivalent to the potassium iodide present in the prepared solvent. The iodine determined by titration is six times the quantity equivalent to the alkali metal. W. R. S.

Determination of very Small Amounts of Bromide. F. L. Hahn. (*Mikrochem.*, 1935, **17**, 222-235.)—It is shown to be theoretically impossible to determine small amounts of bromide in the presence of excess of chloride by oxidation methods depending on the liberation of the bromine. Success may be accidentally obtained by the liberation of some bromine together with an amount

of chlorine equivalent to the bromine left behind. The fluorescein test for bromide in the presence of chloride has been made quantitative. *Reagents.*—(i) *Fluorescein.*—Five g. of the purest fluorescein dissolved in 5 ml. of 0·1 N sodium hydroxide solution and diluted to a litre. (ii) *Buffer solution.*—Approximately N sodium hydroxide and acetic acid solutions mixed in equivalent amounts and $\frac{1}{9}$ to $\frac{1}{10}$ the amount of excess acetic acid to give pH 5·6 to 5·5. (iii) Solution of chloramine-T, 0·01–0·1 molar. (iv) A 5 per cent. solution of sodium hydroxide containing 0·5 per cent. of sodium thiosulphate. The reaction equation is as follows:

$$RH_4 + 4Br' + 4AmCl = RBr_4 + 4AmH + 4Cl'$$

fluorescein   bromide   chloramine   eosine   toluene   chloride
sulphamide

For the determination of amounts up to 2γ of bromine 3 drops of buffer, 1 drop of fluorescein, 1 drop (0·01 molar) of chloramine-T are added to the solution, and the reaction is stopped by means of solution (iv) after 1 to $1\frac{1}{2}$ minutes. As the determination must be carried out at a definite pH, strongly acid or alkaline solutions must be neutralised to methyl red, the colour of which is destroyed under the conditions of the determination. Ammonia interferes and must be removed by evaporation with alkali.

A second method of determination, rapid but less accurate, has been based on Heyrowsky's polarographic method, which itself, however, was not sensitive enough. A mercury-calomel electrode was found to be very sensitive to small amounts of bromide in potassium chloride solution. The *comparative electrode* used is a calomel electrode with lead to a potassium chloride-agar solution, and, when not in use, the lead is dipped in a potassium chloride solution of the same concentration. Two different procedures are described, of which the second is suited for extremely small volumes:—(i) *Liquid electrode.*—About 0·5 to 1 ml. of the test solution is put into a small test-tube through the bottom of which a platinum wire passes, and a few drops of pure mercury are added as electrode, then a drop of a phosphate buffer solution, pH about 6, followed by a drop of 0·01 N of mercurous perchlorate solution. (ii) *Wire electrode.*—An electrode can be constructed of metallic mercury and calomel in an intimate mixture which will change its potential with the bromide-content of potassium chloride solutions if a platinum wire is polarised in a concentrated sublimate solution. The wire can either be dipped in the test solution, or bent round and the drop placed in the bend. A number of determinations of bromide in concentrations varying from 0·05 to 2·0 per cent. gave results differing from the calculated amounts by 0·00 to 0·03 per cent. The method is capable of being made more accurate.   J. W. M.

Note on the Determination of Iodine. B. F. Stimmel and D. R. McCullagh. (*J. Biol. Chem.*, 1936, **116**, 21–24.)—The method, first published in 1934 (*J. Biol. Chem.*, 1934, **107**, 35) has been modified for the micro-determination of iodine in thyroid tissue and blood. For the determination in thyroid tissue, the fresh material, weighing between 5 and 100 mg., is placed in a 70-ml. nickel crucible with 0·5 to 1·0 ml. of a saturated solution of potassium hydroxide. This is heated over a Bunsen flame until the tissue disintegrates and is evenly distributed throughout the hydroxide. The crucible is then placed in a furnace at 100° C.,

and during a period of about 30 minutes the temperature is raised to 400° C., after which the crucible is immediately removed. The fused mass is washed into a 50-ml. Claissen flask with not more than 25 ml. of water. The auxiliary tube of the flask is sealed off, and the side-tube is connected with a condenser, the lower end of which goes to the bottom of a 50-ml. extraction flask which contains just sufficient water to cover the end of the condenser tube, 0·2 ml. of a 3 per cent. solution of sulphuric acid and 0·2 ml. of a 10 per cent. solution of sodium bisulphite. To the contents of the Claissen flask are added quickly 2 ml. of a 50 per cent. solution of sulphuric acid, 1 drop of a 10 per cent. solution of ferric sulphate, and 2 ml. of a 3 per cent. solution of hydrogen peroxide. More acid is added if necessary in order to make the solution definitely acid, as indicated by the presence or absence of ferric hydroxide. A glass bead is added to prevent bumping. The flask is immediately closed with a stopper and dropping funnel, the outlet of which has been drawn to a point. The contents of the flask are boiled vigorously over a micro-burner, care being taken to avoid flooding the side-arm of the Claissen flask. One or two additional amounts of 2 ml. of the 3 per cent. hydrogen peroxide solution are added through the dropping funnel during the distillation, but heating is discontinued during these additions to avoid carrying hydrogen peroxide over with the distillate. The distillation is discontinued when the volume in the Claissen flask is reduced to about 5 ml., or when sulphates begin to crystallise on the side of the flask. The extraction flask is then placed on a wire-gauze having an asbestos centre, and the contents are boiled gently for 2 minutes to expel carbon dioxide and sulphur dioxide, a glass bead being used to prevent bumping. The solution is immediately made alkaline to litmus paper by the addition of a 10 per cent. solution of potassium hydroxide; this should not require more than 3 drops. The solution is then carefully boiled down to a volume of 5 or 6 ml.; 1 drop of methyl orange solution is added, and the solution is neutralised by the addition of a 3 per cent. solution of sulphuric acid. Two drops of the sulphuric acid are added in excess and 5 drops of bromine water, which should cause the solution upon shaking to turn yellow immediately. It is then boiled down very cautiously to about 2 ml. and cooled on ice. This should require between 3 and 5 minutes in order to remove completely the excess of bromine with the minimum loss of iodine. One drop of a 1 per cent. solution of starch and 2 drops of a 1 per cent. solution of potassium iodide are added, and the solution is titrated with a 0·001 N solution of sodium thiosulphate which is delivered from a 0·2 ml. pipette graduated to 0·001 ml.

In determining the amount of iodine in blood, 10 ml. of blood are boiled with 12 ml. of a saturated solution of potassium hydroxide in a 300-ml. nickel crucible until foaming ceases. This requires less than 10 minutes. The crucible is placed in a muffle furnace at 250° C. for 30 minutes. The temperature is then increased to 360° C. over a period of 30 minutes. It is allowed to remain at 360° C. for 10 minutes, after which the crucible is removed. Sufficient water is added to the fused mass to form a fluid paste, which is extracted once with 25 ml. of 95 per cent. ethyl alcohol and four times more with 10 ml. of the same solvent. The combined alcoholic extracts are placed in a 300-ml. nickel crucible with 0·5 ml. of a saturated solution of potassium hydroxide. The alcohol is

evaporated on the steam-bath, the contents of the crucible are gently dried over a free flame, and the crucible is placed in a muffle-furnace at 385° C. for 15 minutes. The ash is then transferred to a 50-ml. Claissen flask, and the procedure described for thyroid tissue is followed.   S. G. S.

The Copper Benzidine Reaction in the presence of Halogen Salts and Thiocyanates, and a new Test for Copper. L. Kuhberg. (*Mikrochem.*, 1936, **20**, 153–160.)—The theories of the copper-benzidine reaction are discussed. For the test to be successful on filter-paper the solutions must be added in the following order: first, the drop of the copper salt solution, then benzidine, and finally the thiocyanate or bromide solution; *o*-toluidine reacts similarly to benzidine. Acetone is the best solvent for the benzidine or *o*-toluidine. Thiocyanates and bromides are used preferably to iodides in the reaction, as iodides form coloured products with a number of cations, and further are too easily oxidised. The *limit of identification* for the benzidine test is 0·08γ copper in 1:16,700 dilution for bromides and 0·01γ copper in 1:1,333,000 dilution for thiocyanates. The *o*-toluidine reaction is even more sensitive. The reagent solution contains 0·1 g. *o*-toluidine and 0·5 g. ammonium thiocyanate in 5 ml. of acetone. Aqueous solutions are unsuitable, as the copper-toluidine complex is somewhat soluble in water. The order of adding the different participants in the reaction is unimportant. When the drops were added from a fine capillary on to filter-paper, first a drop of the toluidine-thiocyanate reagent, then a drop of the test solution, the *limit of identification* was 0·003γ and the *concentration limit* 1:5,000,000. The following ions interfere: silver, univalent mercury, bi- and ter-valent iron, ter-valent thallium, quadri-valent cerium, ter-valent gold, chloroplatinic acid and large amounts of manganese. The interference of silver may be prevented by precipitating it as chloride before the reaction, while iron may be converted into the complex $[FeF_6]'''$ ion by the addition of alkali fluorides, and in that form is without action on toluidine, so that it is possible to detect as impurity 0·00016 per cent. of copper in iron salts. In the presence of manganese a comparison test should be carried out on a saturated solution of manganese sulphate. In order to detect copper in the presence of the above-named oxidising agents and of univalent mercury, the differential reduction with liquid bismuth amalgam is applied, by adding 0·3 ml. of bismuth amalgam to 0·2–0·5 ml. of the test solution and then adding 3 to 4 drops of 0·5 N sulphuric acid. The micro-test-tube is shaken for 1 or 2 minutes, whereby $Tl^{\cdots}$ and $Ce^{\cdots\cdot}$ are reduced to $Tl^{\cdot}$ and $Ce^{\cdots}$, and $Au^{\cdots}$, $PtCl_6''$ and $Hg^{\cdot}$ are reduced to the metallic state. The mixture is filtered or centrifuged and 1 or 2 drops of a 10 per cent. iron ammonium alum solution are added to the filtrate; this completely converts the copper into the bivalent condition. Two or three drops of a saturated solution of sodium fluoride and sodium acetate are added, and the test is then carried out in the usual way. Bivalent tin should be converted to the quadrivalent form before the test. In this way copper may be detected in saturated solutions of the interfering cations.
                                                                J. W. M.

Physical Methods, Apparatus, etc.

Standard Liquids for the Microscopic Determination of Refractive Index. A. H. Kunz and J. Spulnik. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 485.)—The method of determination of refractive index which involves microscopic examination of a particle of the substance in a liquid of known refractive index (in which the particle is insoluble) requires a number of suitable liquids which differ in refractive index by small and regular intervals over the desired range (Chamont and Mason, *Handbook of Chemical Microscopy*, 1930). In view of difficulties in collecting such a set, from pure liquid compounds available, it is proposed to employ mixtures made up to definite values of refractive index as measured by a refractometer. It is desirable that the vapour-pressures of the constituents of a mixture should be nearly the same in order to ensure that the composition remains constant during use. Compounds in the undermentioned list have proved suitable.

Compound	Vapour Pressure mm.	Refractive Index/20° C.
α-Bromonaphthalene	3·5/110° C.	1·6582
n-Butyl phthalate	3·8/110° C.	1·4932
Heptylic acid	1·9/ 90° C.	1·4234
Mesitylene	27·15/ 20° C.	1·4981
Ethyl propionate	27·75/ 20° C.	1·3841

The range between 1·6582 and 1·4234 can be covered by mixtures of α-bromonaphthalene and heptylic acid, and the range between 1·4981 and 1·3841 by mixtures of mesitylene and ethyl propionate; *n*-butyl phthalate can be substituted for the more expensive heptylic acid above 1·4932. S. G. C.

Physico-Chemical (Conductivity) Method for the Determination of Chlorides in Biological Liquids. S. Mihaéloff. (*Bull. Soc. Chim.*, 1936, [5], 3, 2395–2403.)—The liquid to be tested (0·05 to 1 ml.) is measured into the conductivity-vessel, and suitably diluted with water so that the electrodes are covered. The vessel is then warmed (by means of the hand) to a temperature 4° to 5° C. higher than that of the room, and it is maintained at this temperature (within 0·1° C.) by means of the hand during the titration (10 to 12 minutes); 0·5 N to 0·025 N silver nitrate solution is used, according to the amount of chloride present. The pipettes used for measuring the sample are made from glass capillary tubes having an internal cross-section about 0·1 square cm. in area, and they are calibrated by weighing the amounts of mercury which occupy given lengths; the pipetting error is reduced to 1 per cent. if traces of the sample remaining in the pipette are washed out with water, the washings being added to the sample in the cell. Special precautions to avoid errors due to evaporation are also described. Multiplicate determinations made on urine and defibrinated cow and sheep bloods showed that the mean error is less than 0·7 per cent. (corresponding with 0·1 g. of chloride ion per litre) when 0·1 or 0·2 ml. of solution is taken. Agreement with the Mohr, Volhard and persulphate methods was very satisfactory for the 7 urines tested, but the conductivity method gave the best reproducibility, besides being more rapid and requiring a smaller volume of sample. The mean chloride-contents found for whole blood, defibrinated blood and blood serum were 6·3, 6·6 and

6·0 g. per litre, respectively.   Values (in g. per litre) for 15 individuals of either
sex, aged 10 to 47 years, were 5·8 to 7·5 (mean 6·6) for serum, and 5·9 to 16·5
(mean 8·5) for urine.   High results with both were obtained immediately after a
meal, the greatest increases being 0·3 g. per litre for blood and 3·9 for urine.   The
importance of the ratio of the chloride-content of the urine to that of the blood
as a measure of the retention of chlorides characteristic of certain haemo-renal
disorders is emphasised, and it is maintained that this is preferable to the ratio of
the corresponding electrical conductivities because it is a more sensitive indication
and because the latter method is influenced also by the presence of mineral sub-
stances other than chlorides (e.g. sulphates and phosphates) in the blood; moreover,
although the conductivity of serum is proportional to the chloride-content, this
rule does not apply to urine.   The method has also been adapted to the deter-
mination of inorganic conducting elements in cephalo-rachidian fluid.   J. G.

Reviews

POISONS LAW.   A GUIDE TO THE PROVISIONS OF THE PHARMACY AND POISONS
    ACTS, 1852 TO 1933, AND THE DANGEROUS DRUGS ACTS, 1920 TO 1932, FOR
    THE USE OF PHARMACISTS AND OTHERS CONCERNED WITH TRANSACTIONS IN
    DRUGS AND POISONS.   By HUGH N. LINSTEAD.   Pp. 444.   London: The
    Pharmaceutical Press.   1936.   Price 5s. net.

The law relating to poisons, always intricate, has become much more so since
the introduction of the Poisons Act of 1933.   Mr. H. N. Linstead, the able Secretary
of the Pharmaceutical Society, has at the request of his Council written a book
which does much towards reducing these intricacies to simple terms, and as a result
he has produced a work indispensable to the pharmacist and to many others
engaged in the distribution of poisonous substances.   Although written primarily
for the use of the pharmacist, it also contains information which concerns those
engaged in the profession of analytical chemistry.   The book includes chapters
setting out in a precise form the powers and duties of the Pharmaceutical Society,
the history of poison legislation and the responsibilities of the pharmacist; a perusal
of these chapters may be commended to many correspondents whose letters
printed in the ephemeral journals relating to chemistry have in the past shown
much lack of understanding of these responsibilities.

In Mr. Linstead's book there is a skilfully written chapter describing the
various ways in which the Rules and Regulations made under the Poisons and
Dangerous Drugs Acts affect different interests.   It includes the application of
these Acts not only to pharmacists and others engaged in retail distribution, but
also to wholesalers, manufacturers, medical practitioners, hospitals, educational
institutions and laboratories, and this chapter sets forth most clearly under the
separate headings those things which each may or may not do without offending.

The author has been fortunate in securing a remarkably clearly written
chapter by Sir Malcolm Delevingne relating to the Dangerous Drugs Acts, and
explaining with commendable lucidity the history of, and the case for, international
co-operation in suppressing traffic in drugs of addiction.

Approximately one-half of the book—over 200 pages—is devoted to Appendixes. The first of these gives the text of the various Acts, and the various Rules, Regulations and Official Interpretations made under them, and the second gives a list of poisons and substances containing poisons included in the three Schedules to the Rules. The list is very full—it would be impossible for it to be exhaustive— and it gives in a form suitable for ready reference the proportions of poisons permitted in substances falling within the three Schedules.

Owing to the new Act, Rules and Regulations, it has become necessary for analysts to make accurate assays of various alkaloids, glucosides, etc., for which no well-authenticated methods of analysis exist; this need arises in order to determine whether certain preparations belong to Schedules 1, 2 or 3, according to the content of the poisonous substance. For example, preparations of gelsemium root containing 0·1 per cent. and upwards of alkaloid, or those of cantharidin containing 0·01 per cent. and upwards of cantharidin, are to be included in Schedule 1; consequently the accurate estimation of these substances in preparations containing amounts near to the permissible limit has become a matter to which the analyst has had to direct his attention since they came into force. The Analytical Methods Committee of this Society has lost no time in concentrating attention on this matter.

Finally, while the book is not to be regarded as a legal guide, it is an admirable and concise summary of the intricacies of poison legislation. FRANCIS H. CARR

FLAVOURS AND ESSENCES. A HANDBOOK OF FORMULAE. By M. H. GAZAN. Pp. 115. London: Chapman & Hall, Ltd. Price 25s. net.

This book contains over 300 formulae, prepared by a member of a Dutch family closely associated with the flavouring industry, and covers all the commoner fruit essences and many wine and liqueur flavours. The formulae consist mostly of compounds of natural essential oils mixed with various synthetic flavours— chiefly esters and aldehydes—and the author points out that "artificial flavours, however skilfully prepared, always lack the finish that characterises essences containing the natural fruit juice," so that he recommends the addition to the artificial essence of about 45 to 65 per cent. of natural fruit juice.

The formulae are divided into essences, "ethers," basic compounds, and extracts (from leaves, peel, root, etc.), and many of them are somewhat involved, owing to the numerous cross-references; for example, strawberry essence B contains proportions of six other essences, four of which are in turn composed of four or five other essences, and these are again compounded from others, so that to prepare any one essence one must have already prepared very many more. It is quite evident, therefore, that such formulae would be chiefly of value in large establishments where a great variety of essences are manufactured, though useful hints might be obtained by the chemist seeking to analyse or formulate a flavouring essence.

Formulae occupy 96 pages of the book, and the remaining 20 pages consist of an appendix giving a list, with properties, of the principal synthetic flavours, together with the usual methods for determining their specific gravity, boiling-point and melting-point, and also a note on colouring material. This appendix rather detracts from the character of the book, as there are many errors in the

chemistry of the synthetics, and it might well have been omitted.   Thus, acetaldehyde is said to boil at 122° C., benzoic acid to have a specific gravity of 122, sweet spirit of nitre to consist of ethyl *nitrate*, the formula of ethyl phenylacetate is given for phenyl ethyl acetate, and vanillin is said to be prepared by oxidising isoeugenol with alcoholic potash.   In the section on colouring essences, details are given for the quantitative preparation of the concentrated dye solution, and of the quantities of the solution to be used in the various essences, but the nature of the dye itself is not even mentioned.

The book is well printed and attractively bound in leatherette, but its price appears rather high in proportion to its size and contents.   W. H. SIMMONS

PREGL'S QUANTITATIVE ORGANIC MICRO-ANALYSIS.   By HUBERT ROTH.   Third English Edition, translated from the Fourth Revised and Enlarged German Edition by E. BERYL DAW, B.Sc., A.I.C.   Pp. xvi + 271, with 72 illustrations.   London: J. & A. Churchill, Ltd.   1936.   Price 18s.

This is a very welcome translation of the only complete book written on the methods of organic micro-analysis, the German edition of which has already been reviewed in this Journal (ANALYST, 1936, 583).   It is significant, as showing the enormous interest in micro-methods, that this work, first published in 1917, has been through four editions in German, and three in English since 1923.   It is a model of what a textbook should be: it contains complete information, all of it thoroughly tested by experience, but it is a pity that the inadequacy of the index in the German edition could not have been remedied in the English edition.

The translator has done her work carefully and conscientiously.   In her preface she states that she has "endeavoured to follow the original closely," and it might be objected that the result has been a translation that errs in being a trifle too literal.   The book is well printed and is similar in format to the second English edition.   JANET W. MATTHEWS

SEMI-MICRO QUALITATIVE ANALYSIS.   By CARL J. ENGELDER, Ph.D., TOBIAS H. DUNKELBERGER, B.S., and WILLIAM J. SCHILLER, Ph.D.   Pp. x + 265.   New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd.   1936.   Price 13s. 6d. net.

In this work the authors have aimed at applying the conventional methods of separation of the commoner inorganic ions on a semi-micro scale, making use of the technique of drop reactions.   In this new technique the group separations and confirmatory tests are made upon about one ml. of the solution, and if this volume contains 1 mg. of each cation constituent and 5 mg. of the anion constituent, sufficient quantities are left after the separations to apply confirmatory tests, these being carried out on spot-plates or drop-reaction paper.

The book is divided into four parts.   Part I (pp. 9–101) deals with fundamental principles of qualitative analysis, and discusses theories of ionisation, reaction velocity, mass action and many other subjects, including the theories of valency and the structure of atoms.   Part II (pp. 102–177) gives a full account of the methods of separation and identification of the commoner cations.   The group separations follow the well-established classical methods in macro-analysis, one notable change being the incorporation of the fairly recent method of Williams and

Briscoe (*Chem. News*, 1932, **145**, 177) for the separation of calcium from barium and strontium. The underlying theoretical principles are well explained, and a comprehensive account of confirmatory tests for each cation is given, including numerous sensitive tests with organic reagents. In Part III (pp. 178–216), the reactions of the common anions are discussed, and in Part IV (pp. 217–246) a systematic scheme of micro-analysis is given.

The most welcome feature of the book, which will be of particular interest to analysts, is the inclusion of organic spot reagents for cations and anions. The authors have compiled an excellent up-to-date bibliography on the subject, comprising over five hundred references; but one cannot help feeling that some important reagents and tests in common use are either altogether omitted or very briefly mentioned. Thus on p. 121 the use of thiourea, which is a specific reagent for bismuth, is very briefly described, whereas considerable space is devoted to relatively unimportant tests, such as that with sodium stannite. Among some twenty reagents for copper (pp. 122–124), sodium diethyldithiocarbamate, introduced by Callan and Henderson, which is probably one of the most sensitive, is altogether omitted. The Gutzeit test for arsenic, in which, by the way, the authors use silver nitrate paper, is disposed of in eight lines, and no reference at all is made to the Marsh or Reinsch test. Again, all the authors have to say about thioglycollic acid, which is capable of detecting readily 1 part of iron in 5,000,000, is "thioglycollic acid gives a red coloration." An important point upon which the reviewer particularly wishes to lay stress is the necessity of giving *full* details of the interfering ions and the methods of eliminating them in the application of these tests. The use of spot tests has often found disfavour, owing to scant regard being paid to interfering substances. The benzidine test (p. 148) used for manganese is no more than a generic test for salts of a number of metals with oxidising properties.

Sufficient has been said to indicate that if there are to be, as we hope, further editions of this book, it would be useful to revise this subject critically and to give a few well-tried tests with full details of the technique and an adequate account of interference caused by other ions. As is frequently to be observed in similar books coming from America, the theoretical section seems far too long for a book on qualitative analysis. Some reference to theory is certainly an advantage and would, no doubt, be cordially welcomed by the student who wishes to have a thorough grasp of the subject, but inasmuch as a book of this kind is essentially practical, it would be better, perhaps, to keep the theoretical part within more reasonable bounds.

In fairness to the authors it must be said that the scheme which they here present, and which they have tested in teaching practice over a number of years, should commend itself to teachers because of the great economy of material and also because of the powers of observation developed in the pupil. Moreover, the professional analyst will find a wealth of information in the book; he should also derive from it numerous hints as to how to deal with the analysis of small amounts of substances, such as, for example, the ash of a food or drug, or a water residue. Those engaged in research in qualitative analysis in general, and in the use of organic reagents for inorganic analysis in particular, will find the book invaluable. H. R. NAʼJI

TEXTBOOK OF APPLIED BIOCHEMISTRY. By F. C. WOKES, B.Sc., Ph.C., F.I.C. Pp. ix + 522, with 79 illustrations. London: Baillière, Tindall & Cox. 1937. Price 15s.

Sufficient of this book is devoted to descriptions of chemical and bio-chemical analyses, as well as to the theoretical considerations underlying those analyses, to make it of practical interest to readers of this journal, especially to those concerned with certain kinds of pharmacopoeial assay. Quite apart, however, from directly utilitarian value, it possesses special qualities that take it outside the ranks of the ordinary student's text-book. As a survey of modern bio-chemistry it has been so planned and written that it can be followed by all who have been taught chemistry—and have remembered most of what they have been taught. To chemists engaged in industrial or consulting practice—even to some engaged in research—if their work at any time involves contact with pharmaceutical manufacture or the examination of its products, Mr. Wokes has rendered a great service, even though his book was written primarily with the student in mind.

The chapter headings may be enumerated in substantiation of this claim, and not merely as a reviewer's method of padding. The author has with some originality begun by considering "The Biochemical Importance of Water." He then discusses in turn Hydrogen Ion Concentration; Surface Phenomena and Colloids; Spectroscopy; Sources and Properties of Ultra-Violet Light. There follow three chapters on Carbohydrates; Fats; Proteins and other Nitrogenous Compounds. The last four chapters are more pronouncedly biochemical, and contain admirable accounts of Enzymes and Respiration; Bacteria; Hormones; Vitamins. The book ends with an Appendix on International Biological Standards, a list of references and author-index, and a general index.

Any analyst who wants to fill up the gaps in his biochemical knowledge—whether those gaps have ever been filled before or not—will find the necessary material for most of them in this highly interesting and informative volume.

A. L. BACHARACH

SURVEY OF IMPORTS, RAW MATERIALS AND SYNTHETIC PRODUCTS, AND THEIR RELATIONSHIPS TO THE OLD AND NEWER INDUSTRIES, WITH SPECIAL REFERENCE TO THE HUMBER AREA. A. R. TANKARD. Pp. 54. The City Laboratories, Hull. Price 2s. 6d.

The City of Hull is to be congratulated upon this enlightened means of attracting new industries to its neighbourhood. The manufacturer seeking a factory site will find here detailed information as to the materials conveniently obtainable in the Humber area, and the industrial chemist with no such aim will also find the booklet very informative. Ten pages are occupied by a list of materials imported into Hull, and their uses. Industries already established in the neighbourhood furnish a variety of products required in modern chemical manufacture, notably alcohol and a host of organic solvents, the nature and applications of which are set out in Tables and Charts. A condensed, but very lucid, survey of some recent developments in the manufacture of synthetic products, including artificial resins, plastics, emulsifying agents, silicon products, and so forth, occupies nearly half the book. There are no statistics nor quantitative data; the book is well indexed, and in a small compass places a surprising number of modern products in their industrial setting.

J. H. LANE