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Death

WITH deep regret we record the death of William Thomas Burgess, Vice-President in 1918–19.

The Evaluation of Hydrogen Peroxide

BY S. M. TRITTON, M.P.S., F.I.C.

(*Read at the Meeting, April* 5, 1939)

PART I

REAGENTS FOR EVALUATING HYDROGEN PEROXIDE.—Hydrogen peroxide, as supplied for surgical and pharmaceutical purposes, has frequently been found to vary in its efficacy. This has not been found to be due to loss of strength of the peroxide, and it is therefore very likely either that the peroxide has been over-stabilised or that unsuitable stabilisers have been used. It seemed desirable to investigate this point.

The B.P.C. states that "Hydrogen peroxide owes its efficacy as an antiseptic to the readiness with which it liberates oxygen in the presence of living or dead tissues and bacteria," but there appears to exist no method for testing this ease of decomposition. Since catalase is a factor in organic matter that catalyses the decomposition of hydrogen peroxide, with liberation of one atom of oxygen, it was decided to prepare a solution of that enzyme for testing the samples of peroxide examined.

For the estimation of catalase with peroxide Rosenblum[1] suggests (*a*) that the peroxide has to be buffered, and (*b*) that titanous chloride is the only suitable reagent for the titration of peroxide in presence of catalase.

In view of this statement, a number of peroxide preparations were tested by titration with titanous chloride, and the results were checked (1) by titration with potassium permanganate and (2) by titrating with sodium thiosulphate solution the iodine liberated by the peroxide from potassium iodide. In the tests with titanous chloride it was found that 0·5 ml. of a 0·04 per cent. solution of methylene blue gave a very satisfactory end-point if the dyestuff was added near the end of the titration, *i.e.* when the orange colour had faded to pale yellow.

By the courtesy of various manufacturers eight stabilised peroxide preparations and three not stabilised were obtained. No attempt was made to investigate the stabiliser present, apart from titrating the peroxides for acidity and ascertaining whether the stabiliser was organic or inorganic. The results of the tests on these eleven samples are given in Table I.

TABLE I

Hydrogen peroxide preparation (100 ml.)	Acidity to methyl orange, *N*/10 sodium hydroxide required ml.	A Permanganate, *N*/10 ml.	B Thiosulphate, *N*/10 ml.	C Titanous chloride, *N*/10 ml.	Difference A–C ml.
1. Stabilised by inorganic substance	2·4	18·0	17·95	17·35	0·65
		18·0	18·0	17·4	0·6
2. Stabilised by organic substance	4·0	18·5	18·5*	18·4	0·1
		18·5	18·5	18·4	0·1
3. Stabilised by sulphuric acid ..	10·0	15·95	16·0	15·3	0·65
		16·0	16·0	15·3	0·7
4. Stabilised by sulphuric acid ..	10·0	18·8	18·8	18·4	0·4
		18·8	18·8	18·4	0·4
5. Stabilised by inorganic substance	2·4	17·2	17·2	17·0	0·2
		17·2	17·2	17·0	0·2
6. Stabilised by organic substance	neutral	17·7	17·7	17·3	0·4
		17·7	17·7	17·3	0·4
7. Stabilised by organic substance	neutral	17·1	17·1	16·7	0·4
		17·1	17·1	16·7	0·4
8. Stabilised by organic substance	3·2	10·3	10·3*	10·1	0·2
		10·3	10·3	10·1	0·2
9. Not stabilised	2·4	14·7	14·7	14·7	nil
10. Not stabilised	neutral	17·3	17·3	17·3	nil
11. Not stabilised	3·2	19·0	19·0	19·0	nil

* Very slow liberation of iodine.

As is shown in Table I, the results of the permanganate and thiosulphate titrations agreed closely in every test whether the peroxides were stabilised or not. Where the stabiliser was organic and of a weakly acidic character, liberation of iodine was very slow and it was essential to use starch to obtain a satisfactory end-point; otherwise this was not necessary, for the change from yellow to white can be discerned by the eye, but not the slow return of yellow. The results of the titanous chloride titration agreed well with those obtained by titration with permanganate and thiosulphate for the three samples without stabilisers, but were invariably low when stabilisers were present.

It seemed incredible that with the sample (No. 3) in which the stabiliser was known to be sulphuric acid only, there should be this discrepancy between the potassium permanganate and titanous chloride titrations, but the determinations were kindly checked by Mr. J. H. Harwood, B.Sc., A.I.C.

As a further check, 0·05 ml. of 2 *N* sulphuric acid were added to 10 ml. of peroxide No. 10 and the mixture was diluted to 100 ml. There was no difference between the results obtained with permanganate and titanous chloride when the titrations were carried out at once or after 1 hour's standing, but after a week's standing the difference was as much as 1·0 ml. (Table II).

TABLE II

Hydrogen peroxide No. 10	Permanganate, *N*/10 ml.	Titanous chloride, *N*/10 ml.	Difference ml.
10 ml. + 0·05 ml. of 2 *N* sulphuric acid made up to 100 ml. ..	13·0	13·0	0·0
	13·0	13·0	0·0
10 ml. + 0·05 ml. of 2 *N* sulphuric acid made up to 100 ml. after standing 1 week	13·0	12·0	1·0
	13·0	12·0	1·0
10 ml. made up to 100 ml.	13·0	13·0	0·0
10 ml. made up to 100 ml. after standing 1 week	11·4	11·4	0·0

Attempts were made to ascertain if the titration could be completed by addition of permanganate after the required quantity of titanous chloride had been added; the methylene blue indicator was, of course, omitted. The results given in Table III show that this can be done.

TABLE III

	Hydrogen peroxide	Permanganate, *N*/10 ml.	Titanous chloride, *N*/10 ml.	Difference ml.	Additional *N*/10 permanganate ml.
No. 2.	Original titration (as Table I)	18·5	18·4	0·1	—
,,	After 2 months	18·5	17·5	1·0	1·0
No. 3.	Original titration (as Table I)	16·0	15·3	0·7	—
,,	After 2 months	9·8	9·2	0·6	0·6
No. 4.	Original titration (as Table I)	18·8	18·4	0·4	—
,,	After 2 months	18·8	17·8	1·0	1·0

The sample of peroxide No. 2 after two months had not changed in titre to permanganate, but the original difference of 0·1 ml. between the titanous chloride and permanganate results had increased to 1 ml. A second test was made, the requisite quantity of titanous chloride was added, and the titration could be completed with permanganate. As the original permanganate titration agreed with the thiosulphate titration, it is obvious that the excess of permanganate solution, *i.e.* 1 ml. required over the volume of titanous chloride used, cannot be due to the stabiliser as such. The most likely explanation is that the stabiliser and some of the peroxide form a compound which is not easily reducible by titanous chloride, but is oxidised by a strong oxidising agent, such as permanganate. These results should throw some further light on the theory of stabilisation, a subject which is still much under discussion. Peroxide No. 3 had lost in strength, and the

difference between the results of the permanganate and titanous chloride titrations had altered from 0·7 to 0·6 ml. The difference is too small to be of significance. The bottle containing No. 4 had not been opened often, and the sample had not lost strength at all, but here there was a notable increase in difference—from 0·4 ml. to 1 ml.

As titanous chloride is advocated for the standardisation of buffered hydrogen peroxide used in the titration of catalase, tests were carried out on buffered peroxide, B.D.H. Universal buffer and Clarke and Lubs's phosphate buffer being used. It will be seen from Table IV that here again the titanous chloride figures are low.

TABLE IV

Hydrogen peroxide No. 9	Permanganate, $N/10$ ml.	Titanous chloride, $N/10$ ml.	Difference ml.
25 ml. + 100 ml. of B.D.H. buffer, made up to 250 ml. ..	6·75 6·75	5·9	0·85
25 ml. + 100 ml. of Clarke & Lubs's sodium phosphate buffer, made up to 250 ml.	6·0 6·0	5·35	0·65

The preceding tests show therefore that titanous chloride is not a suitable reagent for the evaluation of hydrogen peroxide, but that titration with potassium permanganate and iodine liberation followed by titration with sodium thiosulphate are equally satisfactory. It was decided to use potassium permanganate in the estimation of catalase with hydrogen peroxide, as it was found that, at the strength used, an extract of liver did not decolorise the permanganate solution.

PART II

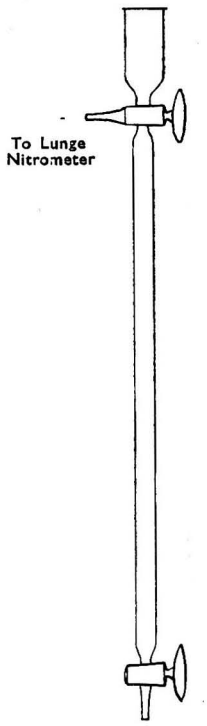
EFFECT OF CATALASE.—It is known that many substances inactivate catalase, notably sulphuric acid, persulphates and pyrogallol. It is reasonable to assume, therefore, that when a stabilised peroxide will not effervesce vigorously with dentures, etc., the stabiliser inactivates the catalase. This was found to occur with peroxides containing acids. With a weak solution of catalase there was hardly any liberation of oxygen. If the solution of catalase was very strong, oxygen could be evolved, despite the presence of acid. This explains why the same peroxide may be effective in the presence of pus, and yet practically useless as a mouthwash or for dentures. If the acid peroxides were first neutralised, inactivation of the catalase was avoided and oxygen was liberated freely, but if alkali was added to the hydrogen peroxide subsequent to the addition of catalase, no further oxygen was evolved, *i.e.* catalase had been permanently inactivated.

For the experiments to be described a solution of catalase was prepared from ½ lb. of fresh, minced calf's liver by leaving it overnight with 500 ml. of chloroform water in a refrigerator and filtering through muslin and filter-paper.

Rosenblum[1] prepared his solution of catalase by treating the aqueous liver extract with an equal volume of 95 per cent. alcohol to remove the proteins, the catalase remaining in solution, while Alyea and Pace[2] precipitated their catalase from solution with 95 per cent. alcohol, and used the precipitate, after purification by dissolving it in water, to obtain a solution of catalase. Although the methods

appear contradictory, they are not so, because actually both the precipitate and the filtrate contain catalase; but I found the filtrate to be unsuitable. It has not been proved whether this is due to insufficiency of catalase or whether alcohol causes inactivation.

It was found that an extract prepared from fresh liver, as described above, gave better results than either of the solutions prepared by alcohol precipitation as just mentioned. It was necessary, however, so to adjust the concentration of the catalase solution that 1 ml., when added to a diluted (1 in 4) peroxide solution, would give a measurable rate of decomposition with most of the peroxides tested. If the catalase solution is very concentrated, even unsuitably stabilised hydrogen peroxides can usually be practically completely decomposed and give quantitative results. The aim here, however, was not to estimate the available oxygen, but to investigate the performance of hydrogen peroxide in presence of organic matter. By making a 1 in 10 solution of the liver extract a suitable catalase test solution was obtained; 0·1 ml. of this solution decomposed 0·03 g. of buffered hydrogen peroxide in 1 hour at laboratory temperature. The hydrogen peroxide was added in excess and back-titrated with permanganate.



METHOD.—Attempts were made to decompose the peroxide with catalase in a Schrödter apparatus and to collect the liberated oxygen over mercury in a Lunge nitrometer, but this was not found satisfactory, as decomposition was most erratic. The use of a narrow-bore tube (capacity about 8 ml.) for the decomposition enabled the reaction to proceed smoothly (Fig. 1). The tube was first evacuated by the use of a Lunge nitrometer containing mercury, and 1 ml. of undiluted peroxide was drawn in. This was followed by 3 ml. of water, with repeated evacuation; by putting the tube under vacuum, practically 1 ml. of catalase solution could be just drawn in when equilibrium with outside pressure was reached. The connection between the reaction tube and the nitrometer was opened, the levelling tube was lowered, and the mercury was kept at the level of the 30-ml. mark of the measuring tube. After 3 minutes, and again after 6 minutes, the connection with the reaction tube was closed for a few seconds while the volume of gas was read. (It is useful to have a half-time period of measuring the volume of gas, as this shows the course of the reaction more plainly.) Table V shows that peroxide preparations, whether stabilised or not, vary enormously in their reaction with organic matter.

Peroxides Nos. 3 and 4, which contained acid, decomposed only partly and very slowly. Nos. 1, 2 and 5 all decomposed quickly with catalase solutions. Nos. 6 and 7, which had good keeping properties, were indeed very suitably stabilised; even with a very weak catalase solution they decomposed with explosive violence. No. 8 decomposed steadily and completely, but rather slowly. No. 9, though not stabilised and of not very high acidity, must have contained traces of substances

which inactivate catalase, possibly persulphuric acid. No. 10 also contained minute traces of inhibitors, but No. 11 decomposed instantaneously, despite an acidity higher than that of No. 9.

TABLE V

THE REACTION OF HYDROGEN PEROXIDE WITH A SOLUTION CONTAINING CATALASE

Hydrogen peroxide preparation (100 ml.) No.	Acidity to methyl orange, *N*/10 sodium hydroxide required ml.	Remarks on the reaction	Volume of oxygen		Volume of available oxygen, by permanganate titration ml.
			After 3 minutes ml.	After 6 minutes ml.	
1	2·4	Quick start, steady evolution	15	19	20·7
2	4·0	Rapid start, 15 ml. in 30 seconds	16	18·5	20·5
3	10·0	Practically no reaction	3	5	11·0
4	10·0	Very slow reaction	4	9	21·0
5	2·4	Very quick start	15	16	18·9
6	neutral	Instantaneous, 20 ml. in 2 seconds	20	20	19·8
7	neutral	Instantaneous, 20 ml. in 2 seconds	20	20	19·9
8	3·2	Rather slow, but consistently steady	8	10	11·5
9	2·4	Very slow and little reaction	5	8·0	8·6
10	Practically neutral	Fairly quick	11	14·5	14·9
11	3·2	Very rapid, 16 ml. in 10 seconds	16	19	21·3

It should be the aim of stabilisation to obtain maximum keeping properties without inhibiting catalase, and a test such as that described above may well be employed when choosing the stabiliser and when testing the suitability of peroxides for pharmaceutical purposes.

I wish to thank Mr. A. Wexler (Hackney Technical Institute) and Dr. P. F. R. Venables (South-East Essex Technical College, Dagenham) for facilities for carrying out this work.

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27, EXETER GARDENS
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The Presence of Leuco-anthocyanins in Criollo Cacao

BY THE LATE A. W. KNAPP, M.SC., F.I.C., AND J. F. HEARNE, A.I.C.

(*Read at the Meeting, May* 3, 1939)

THE range of colours exhibited by sections of cacao seeds of the various botanical varieties is of considerable importance, as there is little doubt that the nature of the coloured bodies present in the original unfermented beans greatly influences the appearance of the manufactured products and is associated with important differences in flavour.

Colours of unfermented cacaos differ very much, and the cotyledons may range from the pure white of the Criollo variety, through the many shades of pink or purple of hybrids, to the deep purple of the Forastero. This distribution of colour appears to be directly related to the botanical variety, although the purple of the Forastero may dominate and conceal an interbreeding with Criollo. Microscopical examination of the cotyledons of unfermented "beans" shows clearly that the purple cells so typical of Forastero beans are quite absent from the pure Criollo variety.

Many investigations of this difference in colour between the two main varieties of cacao have been made, but the complexity of the problem and the unstable nature of many of the constituents of the beans have restricted definite progress. The problem has been further complicated by the fact that many of the earlier investigators directed their attention mainly to Forastero cacao and appear to have been unaware that the fermented beans of commerce show characteristics differing widely from those of the natural unfermented beans. This has led to considerable misunderstanding of the true character of cacao pigments, for during fermentation most of the isolated pigment cells are destroyed, and the diffused and changed purple pigments have been confused with the coloured compounds produced from the catechin and catechu-tannin present in the bean.

Whilst certain of the properties of the colour constituents have been recognised for some time, little has been known of their actual constitution. The position has been clarified by Fincke[1] and by Knapp,[2] who have independently expressed the opinion that the alcohol-soluble pigment of Forastero cacao is an anthocyanin. This view has been confirmed and established by Robinson *et al.*,[3] who report that cacao beans contain cyanidin-3-monoside. No indication of the botanical variety of the beans examined is given in their paper, but as one of us (A. W. K.) selected and submitted them, we are able to state that they were authentic specimens of purple unfermented beans of the species *Forastero amelonado*, and were obtained from the Gold Coast. The exact nature of the sugar residue has yet to be determined. The importance of this new knowledge cannot be over-estimated. It can now be definitely stated that the colour of the fresh cacao bean is due to anthocyanin pigments, and these are no longer to be confused with the tannin and the catechin bodies and their oxidation and condensation products. It should

now be possible to trace this one particular class of pigment substances through the various stages of the fermentation and manufacturing processes.

The genetics of cacao colour have been studied by Wellensiek[4] and by Pound.[5] Wellensiek has shown that the colour of the bean depends on genetic factors, and Pound has attempted to correlate bean colour, pod colour and flush colour, *i.e.* the colour of the young leaves.

With our fuller present knowledge of the anthocyanin character of the Forastero pigments, much more precise botanical investigation of this type should now be possible, and the distribution of colour in the various parts of the plant, such as the flowers, young leaves, pod tissues, etc., can now be studied in greater detail.

As a preliminary step in this direction, a detailed examination of a Criollo pod has therefore been carried out, attention being directed to the various tissues of the pod wall itself, in addition to those of the seeds.

Considerable care has been taken in the selection of the material for examination and with the Criollo cacao advantage was taken of the air-mail services to allow an examination to be made while the pod was in a fresh condition.

Experimental

Work in this laboratory has shown that, on vigorous treatment with strong hydrochloric acid, white Criollo beans yield a solution of brownish-red substances which exhibit in some degree reactions resembling those of the anthocyanins. Fincke[6] has already published a similar observation. The resemblance between these reactions and those of the anthocyanins is, however, of a general nature only, and our experiments have shown that a complex mixture of such coloured bodies exists in these acid solutions.

Solutions of this type have been prepared from the various components of a Criollo pod, and the colour reactions of certain purified pigments have been compared with those of a solution of cyanidin chloride prepared from unfermented Forastero cacao beans.

Forastero Cacao.—Selected Gold Coast pods of the species *Forastero amelonado* were gathered, and the beans were extracted immediately, in order that they should have undergone as little change as possible. An initial attempt was made to dry them directly in the sun, but, owing to the hygroscopic nature of the pulp, the drying was delayed. It was found necessary to wash off much of the adhering pulp before drying could be satisfactorily completed. The beans presented an entirely unfermented appearance. From these, for purposes of comparison, a solution of cyanidin chloride was prepared as follows:

The shell and germs were removed and 20 g. of the coarsely-ground cotyledons were extracted for two hours with 200 ml. of cold 1 per cent. hydrochloric acid. The extract was filtered with the aid of a pressure pump, and the resulting deep red solution was boiled for five minutes with one-quarter of its bulk of conc. hydrochloric acid, in order to hydrolyse the anthocyanin pigment. The mixture was then transferred to a separating funnel, and the liberated cyanidin chloride was extracted with small amounts of amyl alcohol, the total volume of amyl alcohol amounting to 50 ml. This alcoholic solution was withdrawn and repeatedly washed with water and with 1 per cent. aqueous hydrochloric acid. A large excess

of benzene was then added, together with 30 ml. of 1 per cent. hydrochloric acid to take up the precipitated cyanidin chloride. More amyl alcohol was added to extract the pigment from the aqueous acid solution, and the purification processes were repeated. Finally the pigment was taken up in 50 ml. of 0·5 per cent. hydrochloric acid to obtain the solution of cyanidin chloride for use as the basis of reference.

CRIOLLO CACAO.—A pod of the Criollo type, having the "Old Red Ceylon" characteristics, was selected in Ceylon, at our request. The pod was packed in charcoal chips and immediately despatched by air-mail service to England, where it arrived 8 days later.

Little visible change had occurred, and the pod, which appeared to be just ripe, was in good condition on arrival. The beans, which were loosely assembled in the partly dried pulp, were immediately held in water at 90° C. for 10 minutes to destroy the oxidising enzymes and so stabilise the tannin constituents. None of the beans showed any trace of purple; they were all nearly pure white in cross section, and were thus truly representative of the Criollo variety.

Examination of the Criollo Beans.—The "stabilised" beans were shelled, the germs (radicles) were removed, and 20 g. of the coarsely-ground cotyledons were boiled for 30 minutes with 200 ml. of 1 per cent. hydrochloric acid. Little change was observed during the first 10 minutes, the solution remaining quite colourless. Continued boiling, however, resulted in the gradual development of a very pale orange colour. The resulting solution was filtered, sufficient conc. hydrochloric acid was added to increase the acid concentration to 5 per cent., and the solution was further concentrated by boiling. A dark brownish-red solution was quickly obtained, and a quantity of brown material was thrown out of solution.

The mixture was transferred to a separating funnel and extracted with successive small amounts of amyl alcohol, amounting in all to 50 ml. The orange-coloured aqueous layer was withdrawn, and the pigment in the amyl alcohol layer was purified in the manner described above for Forastero cacao. Sufficient 0·5 per cent. hydrochloric acid was finally added to take up the pigment and to give a solution of similar intensity in colour to that of the solution of cyanidin chloride prepared as described above from Forastero cacao.

Comparison was then made between the colour reactions of this purified solution and those of the solution of cyanidin chloride prepared from the Forastero beans. The tests were carried out in the manner outlined by G. M. and R. Robinson[7] for the examination of the anthocyanidins.

These tests included the colours of the solutions in 0·5 per cent. hydrochloric acid, the reactions with sodium acetate, ferric chloride and 10 per cent. sodium hydroxide solution, and the "oxidation" test showing the degree of recovery of the pigment on re-acidification after shaking with air and 10 per cent. sodium hydroxide solution.

Other solutions were prepared by re-precipitating the pigments from the solution in 0·5 per cent. hydrochloric acid, and re-dissolving them in 0·1 per cent. hydrochloric acid, and the colour reactions of these solutions were further characterised by a study of the colours given in solutions of graded pH values, as suggested by Robertson and Robinson.[8]

Examination of other Components of the Criollo Pod.—A similar development of brownish-red bodies was observed on prolonged boiling of other parts of the pod, such as the inner pod wall, the seed coatings, etc., with 10 per cent. hydrochloric acid. Each component was therefore examined in detail, the initial extraction in each case being as follows:

(*a*) *The seed coatings.*—The seed coatings were removed from the cotyledons, swilled with water to remove extraneous pulp and pulp juices, and extracted with boiling 1 per cent. hydrochloric acid. Little colour developed during this first extraction, but, on increasing the acid-content of the filtered extract to 5 per cent. and boiling for 15 minutes, brownish-red substances were produced.

(*b*) *The radicle.*—The amount of material furnished by the white "germs" of all the beans in the pod was so small that it was not possible to carry out so detailed an examination as with the other components. For this reason the preliminary extraction with 1 per cent. hydrochloric acid was omitted, and a direct extraction with 20 ml. of 15 per cent. hydrochloric acid was made. The red-brown pigments were quickly obtained and extracted directly with 20 ml. of amyl alcohol.

(*c*) *The placenta.*—The whole placenta was air dried, coarsely ground and extracted in the same way as the seed coatings.

(*d*) *The inner membrane of the pod wall.*—The soft pulpy lining of the pod wall was peeled off and extracted directly with 1 per cent. hydrochloric acid. Although this material was greyish, with a trace of purple, the extracts obtained on boiling with the 1 per cent. hydrochloric acid were almost colourless. After filtration the acid concentration was increased to 5 per cent., and the solution was boiled until the deep brownish-red colour had fully developed.

(*e*) *The pod wall.*—Sections of the inner portions of the soft yellowish-white pod wall were carefully removed and cut into thin slices, and 20 g. of this material were extracted for 30 minutes with 200 ml. of boiling 1 per cent. hydrochloric acid. The solution, which at this stage had a very pale orange colour, was filtered with difficulty, and the acid concentration was increased as before to develop the red substances.

Each of the coloured acid solutions so obtained was then extracted with amyl alcohol, and the pigments in the alcoholic solution were purified as previously described. Finally the pigment was dissolved in 0·5 per cent. hydrochloric acid, and the colour reactions were observed.

(*f*) *The coloured external skin of the pod.*—From our previous experience with Forastero pods it had been expected that on direct extraction with cold dilute hydrochloric acid, the red outer skin of the Criollo pod would yield a solution of anthocyanin colour. This, however, was not found to be so with this particular pod.

SUMMARY OF RESULTS.—The solution of the purified pigment extracted from the cotyledons of the unfermented Criollo beans gave colour reactions identical with those given by the solution of cyanidin chloride prepared, as described above,

from Forastero cacao. This agreement in colour reactions also applied to the purified pigments obtained from the seed coatings, the radicles, the placenta and the inner lining of the pod wall.

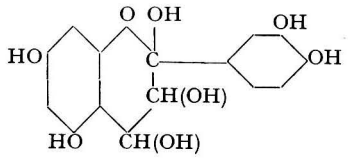
The reactions exhibited by the pigment isolated from the pod wall closely resembled those of the cyanidin chloride solution from the Forastero beans, although certain differences were observed in the ferric chloride reactions. It was thought however, that these slight discrepancies were probably due to the presence of contaminating substances which had not been removed during the purification process, rather than to any difference in the pigments themselves.

Each component of the Criollo pod examined contained, therefore, colourless bodies which yielded, on vigorous treatment with strong hydrochloric acid, a solution containing a mixture of brownish-red bodies. From each of these mixtures, with the possible exception of that from the main pod wall, a purified solution of cyanidin chloride has been separated.

The nature of the other coloured substances developed by the strong acid treatment has not been determined. They are not, however, anthocyanin pigments.

CONCLUSION.—*The Nature of the Colourless Precursor of the Anthocyanin Pigment.*—The experiments detailed above have shown that in practically all the parts of the seed and fruit of Criollo cacao there are present certain colourless substances which may be extracted with 1 per cent. hydrochloric acid to yield practically colourless solutions. On increasing the acid-content, either directly or by prolonged boiling, brownish-red bodies are developed, one of which has been shown to be cyanidin chloride. These facts indicate that Criollo cacao contains a leuco-anthocyanin which, on treatment with strong acid, yields cyanidin chloride.

The nature of the leuco-anthocyanins has been studied by G. M. and R. Robinson,[9] who suggest that the immediate precursor of cyanidin, as shown in formula I, is a ring structure tautomeric with that shown in formula II.



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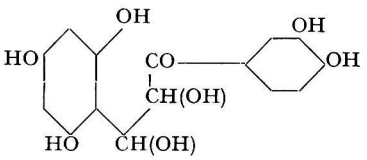

II

It is probable that these leuco bodies in the various tissues of the Criollo cacao pod are present in the form of glycosides.

This discovery may be of use to botanists, especially if the proportionate amounts of leuco-anthocyanin and anthocyanin are related to the extent of inter-breeding of Criollo and Forastero varieties.

We wish to thank Mr. H. Nicholas, of the Gold Coast Agricultural Department, for preparing the Forastero beans, and the Anglo-Ceylon and General Estates Co., Kandy, for the Criollo pod. We also thank Messrs. Cadbury Brothers for permission to publish this paper.

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CHEMISTS' DEPARTMENT
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January 18th, 1939

Discussion

Professor HILDITCH remarked that he had been very interested in Mr. Hearne's description, and asked if colourless leuco-anthocyanins had been isolated in a purified form? He was interested in the possibility of natural leuco-anthocyanins possessing antioxidant properties, since it had recently been observed that concentrates of natural antioxygenic compounds prepared from seed endosperms were basic in character, and lost their power of retarding oxidation in fats after they had been neutralised with a mineral acid.

Mrs. TRITTON asked if the cyanidin chloride could be obtained from the beans in a crystalline state. Also, was it possible to make a quantitative colorimetric estimation of the anthocyanins?

Dr. J. G. A. GRIFFITHS asked if the identity of cyanidin chloride with the product of hydrolysis from Criollo beans had been established by spectroscopic examination.

Dr. H. E. COX expressed surprise at the extraordinary colour change shown to occur between *p*H 12 and *p*H 13 and enquired the cause.

Mr. HEARNE, replying, said that he had not actually isolated leuco-anthocyanins in a pure form. From the fact that the cacao butter itself in chocolate was a particularly stable fat he thought that one might infer that antioxidant substances were present. He had not prepared pure cyanidin chloride from Forastero beans. So far as he knew, the method suggested by Mrs. Tritton for the direct colorimetric estimation of the purple pigment might be feasible, but it had not been found practicable in this particular instance. Spectroscopic methods of examination had not been tried. In reply to Dr. Cox the author said that apparently when *p*H 12 was passed some decomposition occurred and there was a pronounced change; moreover, beyond that *p*H value the colour was not stable.

Studies in the Analytical Chemistry of Tungsten: I

By D. A. LAMBIE, B.Sc., A.I.C.

Although tungsten is one of the best known and most widely used of the "rarer" elements, its analytical chemistry is a difficult subject, which still leaves room for a great deal of improvement. This is due to the idiosyncrasies of the element, *e.g.* its power of complex-formation with a number of elements; it differs from molybdenum and the heavy metals in not reacting quantitatively with the sulphide ion, and from the "earths," in not being precipitated from organic acid solution by tannin or cupferron. This behaviour may be modified by its association with other elements, induced partial precipitation taking place. The detection of small quantities of tungsten and the polytungstic and heteropolytungstic acids also offer a promising field for further analytical investigation.

Having from time to time encountered difficulties in my practical experience of tungsten analysis, I have set myself the task of critically surveying the subject, and of communicating such results as may be of interest or practical value in a series of papers under the above general title.

I. The Recovery of Tungsten from Sulphate Solutions

The recovery of tungsten from sulphate solutions is of great practical interest in its bearing on the separation of tungsten from molybdenum. The most reliable and most convenient method for effecting the separation consists in the precipitation of molybdenum sulphide from tartrate solution, tungsten passing into the filtrate. The quantitative recovery of tungsten from the tartrate solution, however, is admittedly not so simple as that of molybdenum.[1] It involves removal of the tartaric acid, and this is most readily effected by heating with nitric and sulphuric acids; the product is a solution containing sulphuric acid and sodium sulphate (from the alkali added prior to the sulphide precipitation). It is the recovery of tungsten from such solutions that formed the object of this investigation.

The following are the recognised precipitants for tungstic acid: (1) mineral acids, (2) mercurous nitrate, (3) organic bases or alkaloids, and (4) tannin and cinchonine. It was decided to study the effect of sulphuric acid and alkali sulphate on procedures utilising these reagents.

(1) Mineral Acids.—Alkali sulphate is known to have an adverse effect on the precipitation of tungstic acid; thus Hillebrand and Lundell[2] state that "alkali salts prevent complete precipitation of tungsten by digestion with acids." I ascertained that a melt containing 0·05 g. of tungstic oxide, 0·5 g. of potassium bisulphate, and 5 ml. of strong sulphuric acid, when leached with water, gave a faintly turbid solution, which cleared on boiling. This confirms the observations of Powell, Schoeller and Jahn,[3] who reject bisulphate as a means for separating titanium from tungsten. With 0·1 g. of tungstic oxide, 1 g. of bisulphate and 10 ml. of strong sulphuric acid the solution was initially turbid; continued boiling caused hydrolysis, a flocculent yellow precipitate being formed.

Two solutions of sodium tungstate were prepared from carefully purified tungstic oxide, 0·5010 g. and 0·5001 g. being heated with 1 g. portions of sodium carbonate and a few ml. of water in porcelain dishes. The solutions were made up to 500 ml., and the tungstic oxide was determined in 20-ml. portions by (1) precipitation with hydrochloric acid and cinchonine, and (2) Schoeller and Jahn's tannin-cinchonine method.[4]

Solution A:	Method (1).	Found	0·0201 g.	Calculated	0·02004 g.
,, A:	,, (2).	,,	0·0202 g.	,,	0·02004 g.
,, B:	,, (2).	,,	0·0200 g.	,,	0·0200 g.

The values adopted were 0·0201 g. and 0·0200 g., respectively.

The use of sulphuric acid as a precipitant was first investigated. Aliquot portions of sodium tungstate solution were taken in 600-ml. beakers, and varying amounts of strong sulphuric acid and anhydrous sodium sulphate were added. In Expts. Nos. 1 and 2 Margueritte's method[5] was used; the mixture was evaporated until copious fumes of sulphuric acid were evolved, 3 ml. of strong nitric acid were added to re-oxidise any reduced tungstic oxide, and the mass was treated with 40 ml. of water. In Expts. Nos. 3 to 5, after evaporation until sulphuric acid fumes appeared and oxidation with a few drops of nitric acid, the cold mass was taken up with 200 ml. of water and the solution was digested for an hour at boiling-point on a hot plate. In every instance the precipitate was allowed to stand overnight before filtration. The precipitated tungstic acid was collected on a filter-paper of fine texture, having in its apex a pad of filter-pulp, and then washed with 5 per cent. v/v sulphuric acid, ignited and weighed. Without exception the filtrates were clear and remained so on standing. The results are given in Table I.

TABLE I

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.	Added
1	0·0201	0·0190	−0·0011	10 ml. of sulphuric acid
2	0·0201	0·0185	−0·0016	10 ,, ,, ,, ,, and 5 g. of sodium sulphate
3	0·0200	0·0190	−0·0010	10 ,, ,, ,, ,,
4	0·0200	0·0183	−0·0017	10 ,, ,, ,, ,, and 10 g. of sodium sulphate
5	0·0020	nil	−0·0020	10 ,, ,, ,, ,, ,, 5 g. ,, ,, ,,

These results show that the use of sulphuric acid as a precipitant leads to negative errors, which may be proportionately large with small amounts of tungstic acid. As was expected, the presence of alkali sulphate increased the magnitude of the negative error.

Experiments were next made with *aqua regia* as precipitant. Aliquot portions of the sodium tungstate solution were taken in 600-ml. beakers, and varying quantities of sulphuric acid and of anhydrous sodium sulphate, dissolved in a little water, were added. The solutions, measuring about 100 ml., were treated with 40 ml. of strong hydrochloric acid and 15 ml. of strong nitric acid and evaporated to about 50 ml.; five ml. more of strong nitric acid was added and the volume was reduced to 5 to 10 ml. With the larger amounts of sodium sulphate, evaporation was interrupted when the volume had reached 10 to 15 ml. to avoid loss by spurting. Hot water was added to bring the volume to 150 ml., and the solution was heated for half-an-hour on a hot plate.

After standing overnight the precipitated tungstic acid was collected, washed with 1 per cent. v/v hydrochloric acid, ignited and weighed. The filtrates were perfectly clear. In Expts. Nos. 6 to 10 (Table II) the recovery was incomplete. In 11 and 12, an attempt was made to recover the tungsten by adding 5 ml. of 5 per cent. cinchonine solution after the dilution with water, but all the tungstic acid remained in solution.

TABLE II

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.	Added
6	0·0200	0·0195	−0·0005	5 g. of sodium sulphate
7	0·0200	0·0195	−0·0005	10 ,, ,, ,, ,,
8	0·0200	0·0155	−0·0045	20 ,, ,, ,, ,,
9	0·0200	0·0191	−0·0009	10 ,, ,, ,, ,, and 5 ml. of sulphuric acid
10	0·0020	0·0004	−0·0016	5 ,, ,, ,, ,,
11	0·0020	nil	−0·0020	5 ,, ,, ,, ,, Cinchonine added after dilution
12	0·0020	nil	−0·0020	10 ,, ,, ,, ,, Cinchonine added after dilution
13	0·0020	nil	−0·0020	10 ml. of sulphuric acid after fusion with potassium bisulphate
14	0·0230	0·0224	−0·0006	10 ml. of sulphuric acid after fusion with potassium bisulphate
15	0·0524	0·0517	−0·0007	10 ml. of sulphuric acid after fusion with potassium bisulphate
16	0·1023	0·1016	−0·0007	10 ml. of sulphuric acid after fusion with potassium bisulphate

In Expts. Nos. 13 to 16, tungstic oxide was fused in a vitreosil crucible with 0·5 g. of potassium bisulphate, and the cooled melt was re-heated with 10 ml. of strong sulphuric acid until fluid. The solution, when cold, was transferred to a 600-ml. beaker containing water, and the crucible was rinsed with water. The solution (about 200 ml.) was boiled for half-an-hour to precipitate the tungstic acid, filter-pulp and 5 ml. of 5 per cent. cinchonine solution being added. After standing overnight the precipitate was collected, ignited and weighed as before.

These results demonstrate that acid precipitation as a general method for the recovery of tungstic acid from sulphate solution is unreliable even with the addition of cinchonine, as variable negative errors are always incurred.

(2) MERCUROUS NITRATE.—When tungstic acid is precipitated by mercurous nitrate from sulphate solution, mercurous sulphate is co-precipitated, hence a large excess of precipitant is required; the precipitate becomes inconveniently bulky and tends to retain alkali, which contaminates the ignited oxide. Moreover, the ignition of large precipitates of mercury salt is a risky operation. These considerations justify the rejection of mercurous nitrate as a precipitant for tungstic acid from sulphate solutions.

(3) ORGANIC BASES AND ALKALOIDS.—The insolubility of benzidine sulphate renders that base unsuitable for the present purpose.

Cinchonine is by far the most widely used organic precipitant for tungsten; but it has always been recognised that, in presence of alkali salt, complete precipitation occurs only after long standing (Cremer gives 48 hours[6]). It was to be expected, therefore, and has been fully confirmed by Expts. Nos. 17 to 21 (Table III), that alkali sulphate prevents complete precipitation by cinchonine.

In Expt. No. 17, an aliquot volume of sodium tungstate solution was taken, whilst in Expts. Nos. 18 to 21, tungstic oxide was fused with 0·5 g. of potassium bisulphate and the quantity of sulphuric acid given in the Table; the fused mass

was leached, and the solution was treated with excess of cinchonine reagent and set aside overnight before filtration.

TABLE III

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.	Conditions
17	0·0020	0·0008	−0·0012	10 g. of sodium sulphate added
18	0·0113	0·0089	−0·0024	Re-fused with 5 ml. of sulphuric acid
19	0·0236	0·0221	−0·0015	,, ,, 5 ,, ,, ,, ,,
20	0·0215	not weighed	—	,, ,, 10 ,, ,, ,, ,,
21	0·0120	,, ,,	—	,, ,, 10 ,, ,, ,, ,,

In Expts. Nos. 20 and 21, the solution of the melt was clear. Addition of cinchonine produced no precipitate, and even after a week's standing only a very small precipitate was formed, hence these tests were discarded. Two tests made with other alkaloids (quinine and nicotine) proved discouraging, so that this line of investigation was abandoned.

(4) TANNIN AND CINCHONINE.—This method was developed by Schoeller and Jahn[4] to recover small amounts of tungstic acid from solutions containing considerable amounts of alkali chloride which, far from being inimical to the precipitation, was found to assist flocculation.

In Expts. Nos. 24 to 30 (Table IV) aliquot portions of sodium tungstate solution were taken, and varying amounts of sodium sulphate or sulphuric acid were added. The solution, diluted to about 200 ml., was made feebly alkaline with ammonia, heated to 50° C. and treated with a solution of 0·5 g. of tannin. Dilute hydrochloric acid was then added until the solution was acid to litmus, and pulped filter-paper and 5 ml. of 5 per cent. cinchonine solution were stirred in, the stirring being prolonged until a thorough incorporation of precipitate and pulp was achieved. The beaker was set aside to cool (in running water to expedite cooling), and the precipitate was collected on a Whatman No. 41 filter, washed thoroughly with ammonium chloride solution containing cinchonine, ignited and weighed.

In Expts. Nos. 31 to 33, the tannin-cinchonine method was applied to the filtrates from the precipitates obtained in Expts. Nos. 3, 4 and 9 (Tables I and II); the entries under "Tungstic oxide taken" reproduce the negative errors incurred in the earlier experiments. As will be seen under "Tungstic oxide found," recovery was complete.

TABLE IV

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.	Added
24	0·0201	0·0203	+0·0002	20 g. of sodium sulphate and 10 g. of ammonium chloride
25	0·0201	0·0202	+0·0001	40 g. of sodium sulphate and 10 g. of ammonium chloride
26	0·0020	0·0020	nil	——
27	0·0020	0·0021	+0·0001	40 g. of sodium sulphate
28	0·0020	0·0020	nil	20 ml. of sulphuric acid
29	0·0200	0·0202	+0·0002	40 g. of sodium sulphate
30	0·0200	0·0203	+0·0003	20 ml. of sulphuric acid
31	0·0010	0·0011	+0·0001	——
32	0·0017	0·0018	+0·0001	——
33	0·0009	0·0015	+0·0006	——

A few experiments were made to determine the applicability of this method to the recovery of tungstic acid from the solution of a potassium bisulphate melt in ammonium carbonate.

Tungstic oxide was fused with ten times its weight of potassium bisulphate in a vitreosil crucible. The cold melt was leached with 1 g. of ammonium carbonate dissolved in a little water, the crucible being warmed gently on the hot plate to facilitate solution. The liquid was transferred to a beaker, 50 ml. of 20 per cent. ammonium chloride solution were added, and the volume was made up to about 200 ml.; after heating to about 50° C., 0·5 g. of tannin dissolved in a few ml. of water was added (for more than 0·05 g. of tungstic oxide a weight of tannin equal to ten times that of the tungstic oxide was used), followed by dilute hydrochloric acid until the solution was acid to litmus. A cream of pulped filter-paper and 5 ml. of 5 per cent. cinchonine solution added drop-wise were stirred in, and the determination was completed as in the previous series. The results are given in Table V.

In the first two tests (34 and 35) the solutions were filtered hot (35 after standing for two hours on the boiling water-bath), and, as will be seen, gave negative errors. This was no doubt due to the cinchonine tannate remaining in solution at higher temperatures, and so failing to cause precipitation of the colloidal tungsten-tannin complex.

The other tests, in which the solution was allowed to stand until cold before filtration, showed good recovery.

TABLE V

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.
34	0·0065	0·0056	−0·0009
35	0·1014	0·0996	−0·0018
36	0·0256	0·0254	−0·0002
37	0·1053	0·1052	−0·0001
38	0·0518	0·0516	−0·0002
39	0·0109	0·0108	−0·0001
40	0·0050	0·0051	+0·0001
41	0·1000	0·0998	−0·0002
42	0·0506	0·0508	+0·0002

In a further series of experiments (Table VI) the bisulphate melt was leached with water, the crucible was rinsed with dilute ammonia, and the solution was rendered alkaline with ammonia. From this point the precipitation was carried out as in the preceding tests. In Expts. Nos. 53 to 59, after fusion with potassium bisulphate, the cold melt was re-fused with 10 ml. of strong sulphuric acid. This melt, containing a large excess of sulphuric acid, was leached with water and ammonia to give an alkaline solution, and the tungstic acid was precipitated as described above.

The experiments provide ample evidence that the tannin-cinchonine method achieves a quantitative recovery of tungstic acid from solutions containing considerable amounts of alkali sulphate.

It is possible by the tannin-cinchonine method to treat solutions containing not more than 0·2 g. of tungstic oxide. Larger amounts of tungstic oxide would result in inconveniently large precipitates, with attendant difficulties of filtration and washing. Under such conditions one of two alternative procedures may be adopted:—(*a*) the solution may be made up to a convenient volume and an aliquot portion taken containing less than 0·2 g. of tungstic oxide; (*b*) the bulk of the tungstic acid may be precipitated by means of acid, and the balance recovered from solution, after filtration, by the tannin-cinchonine method.

TABLE VI

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.
46	0·0213	0·0216	+0·0003
47	0·0527	0·0528	+0·0001
48	0·1031	0·1028	−0·0003
49	0·0128	0·0129	+0·0001
50	0·0256	0·0255	−0·0001
51	0·0008	0·0011	+0·0003
52	0·0028	0·0031	+0·0003
53	0·0013	0·0016	+0·0003
54	0·1006	0·1004	−0·0002
55	0·1015	0·1012	−0·0003
56	0·1006	0·1007	+0·0001
57	0·0512	0·0518	+0·0006
58	0·0512	0·0514	+0·0002
59	0·1016	0·1018	+0·0002

In order to explore the possibility of combining these last two operations, *i.e.* acid precipitation of the major and tannin-cinchonine precipitation of the minor fractions, three series of tests were made.

A. Effect of excess of acid on the precipitation of tungstic acid by tannin and cinchonine.—Aliquot portions of a sodium tungstate solution were taken, treated with 50 ml. of 20 per cent. ammonium chloride solution, diluted to 200 ml., and heated to about 50° C. A solution of 0·5 g. of tannin in a little water was added and followed by the stated volume of hydrochloric acid, filter-pulp, and 5 ml. of 5 per cent. cinchonine solution. When cold the precipitate was collected, washed, ignited and weighed.

The results recorded in Table VII (Expts. Nos. 60 to 65) show that complete precipitation of tungstic acid takes place in presence of an excess of 10 ml. of strong hydrochloric acid in a volume of 200 ml.

TABLE VII

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.	Strong hydrochloric acid added ml.
60	0·0501	0·0503	+0·0002	1
61	0·0501	0·0500	−0·0001	5
62	0·0501	0·0503	+0·0002	10
63	0·0501	0·0498	−0·0003	20
64	0·0501	0·0496	−0·0005	20
65	0·0501	0·0491	−0·0010	30

In the form in which the method was published by Schoeller and Jahn, the tannin is added to an alkaline tungstate solution which is then acidified. To render alkaline a solution containing a considerable quantity of precipitated tungstic acid would defeat the purpose of the acid precipitation; hence it was of interest to study the effect of adding tannin to the acid solution of tungstic acid.

That an essential difference exists is immediately apparent, for, on treating an alkaline tungstate solution with tannin followed by an excess of acid, a brown colour, or, with more than a few milligrams of tungstic acid a brown flocculent precipitate, results; on the other hand, if tannin is added to the clear acid solution containing even so much as 0·05 g. of tungstic acid, very little colour and no precipitate are produced (such a solution is obtained when 0·05 g. of tungstic acid is fused with 0·5 g. of potassium bisulphate and 5 ml. of strong sulphuric acid, the melt leached with water and the solution boiled). This fact should be borne in mind when tannin is used as a qualitative reagent for tungsten.

B. Tannin-cinchonine precipitation from acid solution.—A number of tests were made in which varying amounts of tungstic oxide were fused with potassium bisulphate and then re-melted with strong sulphuric acid. The final melt, which in general remained clear, was transferred to a beaker containing 100 ml. of water, the crucible was rinsed out, and the rinsings were added to the main solution. A clear solution was obtained on heating, except in the tests with 0·05 g. and 0·10 g. of tungstic oxide. The solution was diluted to 200 ml., heated to about 50° C., and treated with 0·5 g. of tannin dissolved in water, filter-pulp and (dropwise) 5 ml. of 5 per cent. cinchonine solution during vigorous stirring. Addition of the tannin produced no precipitate or change in colour, but on addition of the cinchonine a characteristic buff-brown precipitate was obtained immediately.

After cooling, the precipitate was filtered off, washed as before, ignited and weighed. In Expts. Nos. 66 to 70 (Table VIII) 5 ml. of strong sulphuric acid were added to the melt, and the recovery of tungstic acid was satisfactory regardless of whether ammonium chloride was added or not. With 10 ml. of strong sulphuric acid, however (Expts. Nos. 71 to 76), the recovery was not quite quantitative.

TABLE VIII

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.	Sulphuric acid added ml.	Ammonium chloride added g.
66	0·0503	0·0501	−0·0002	5	10
67	0·0047	0·0047	nil	5	none
68	0·0051	0·0052	+0·0001	5	10
69	0·0109	0·0109	nil	5	10
70	0·0121	0·0120	−0·0001	5	none
71	0·0517	0·0505	−0·0012	10	,,
72	0·0239	0·0233	−0·0006	10	,,
73	0·0008	0·0007	−0·0001	10	,,
74	0·0085	0·0083	−0·0002	10	,,
75	0·0212	0·0204	−0·0008	10	,,
76	0·0538	0·0529	−0·0009	10	,,

C. Combination of acid and tannin-cinchonine precipitations.—It was found that by boiling a solution such as was obtained in Expts. Nos. 71 to 76 (*i.e.*

containing 10 ml. of strong sulphuric acid) the bulk of the tungstic acid was precipitated if more than 0·02 g. was present.

Similar solutions were taken in Expts. Nos. 77 to 80 and boiled for half-an-hour, and the precipitation was completed with tannin and cinchonine, as described under B. When cold, the precipitate was collected, washed, ignited and weighed.

TABLE IX

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error g.
77	0·0202	0·0204	+0·0002
78	0·0528	0·0524	−0·0004
79	0·1032	0·1031	−0·0001
80	0·1030	0·1025	−0·0005

These three series of experiments prove that the precipitation with acid and tannin may be combined, provided that the final solution is not too acid. To verify this conclusion, a final series of experiments was made. In each test 0·5 g. of tungstic oxide was dissolved by heating with 1 g. of sodium carbonate and a few ml. of water. The resulting solution was treated in a 600-ml. beaker with the salts shown in Table X. The volume of the liquid was reduced to 30 or 40 ml. by heating on the hot plate, care being taken to avoid spurting when crystals separated; 30 ml. of strong hydrochloric acid were added, and the solution was boiled for a few minutes to precipitate yellow tungstic acid. The volume was brought to about 300 ml. by addition of hot water, and a solution of 0·5 g. of tannin together with filter-pulp, and 5 ml. of 5 per cent. cinchonine solution were added during thorough agitation. After cooling, the precipitate was collected on a filter of close texture, having filter-pulp in the apex, washed with the ammonium chloride and cinchonine solution, ignited and weighed. The tabulated results (Expts. Nos. 81 to 85) show a satisfactory recovery.

TABLE X

Expt. No.	Tungstic oxide taken g.	Tungstic oxide found g.	Error. g.	Added
81	0·5003	0·5004	+0·0001	5 g. of sodium sulphate
82	0·5013	0·5018	+0·0005	20 ,, ,, ,, ,,
83	0·5007	0·5011	+0·0004	20 ,, ,, ammonium sulphate
84	0·5013	0·5012	−0·0001	20 ,, ,, ,, ,,
85	0·5018	0·5115	−0·0003	20 ,, ,, sodium chloride

SUMMARISED DIRECTIONS FOR THE PRECIPITATION OF TUNGSTIC ACID.—The following technique was adopted in the course of several hundred tungsten determinations. In most instances the ore, which was free from interfering substances such as molybdenum, was decomposed with *aqua regia* to give a residue of tungstic acid and gangue. Treatment of this washed residue with ammonia (or sodium hydroxide followed by excess of ammonium chloride) and filtration provided a solution to which the tannin-cinchonine method could be applied.

Reagents.—Cinchonine solution: prepared by dissolving 50 g. of the alkaloid in 500 ml. of 1:1 hydrochloric acid, diluting to 1 litre with water and filtering.

Ammonium chloride and cinchonine washing solution:—100 ml. of a 20 per cent. ammonium chloride solution and 20 ml. of the above cinchonine solution are diluted to 1 litre with water.

I. In solutions containing not more than 0·2 g. of tungstic oxide.—Unless already alkaline, the solution is rendered so by addition of ammonium hydroxide and 50 ml. of 20 per cent. ammonium chloride solution are added, although this may be omitted if sufficient salts are already present. The solution is diluted to 200 ml., heated to about 50° C., and treated with a solution of 0·5 to 2 g. of tannin in a few ml. of water (tannin at least ten times the estimated weight of tungstic oxide should be added). Hydrochloric acid (1:1) is added, during agitation, until the solution is acid to litmus paper. The creamed pulp of half of a 9-cm. Whatman No. 41 filter-paper is added, followed by 5 ml. of the cinchonine solution added dropwise with continual stirring so as to incorporate the precipitated tungsten-tannin complex, filter-fibre and cinchonine tannate thoroughly. The beaker is set aside until cold or cooled in running water.

The precipitate is collected on a filter-paper of loose texture (Whatman No. 41) and washed three or four times with the ammonium chloride and cinchonine solution, the precipitate being churned up with the stream of liquid. The precipitate is returned to the beaker with the aid of a stream of wash-solution directed into the inverted funnel, stirred up with some wash-solution and re-filtered on the same paper, being transferred completely to the filter, and the washing is completed.

The filter and precipitate are ignited wet in a tared porcelain crucible, the final ignition being carried out over a Bunsen burner to give tungstic oxide, which should be of a good yellow colour. A green residue, which adheres to the crucible, may be obtained if, owing to incomplete washing of the precipitate, alkali salts are still present.

II. In solutions containing more than 0·2 g. of tungstic oxide.—An aliquot portion of the solution may be taken and the tungstic oxide precipitated as under *I.*

If it is desired to precipitate all the tungstic acid, the feebly acid or alkaline solution is cautiously evaporated to 30 or 40 ml. on a hot plate; 30 ml. of strong hydrochloric acid are added, and the solution is boiled so that the precipitated tungstic acid turns yellow. The solution is diluted to 300 ml. with hot water and treated with 0·5 g. of tannin dissolved in a little water, filter-pulp as described above, and 5 ml. of cinchonine solution added dropwise with vigorous stirring.

When cold, the liquid is filtered through a filter-paper of close texture (Whatman No. 40) having a pad of filter-pulp in the apex. The washing is carried out with ammonium chloride and cinchonine solution as in the previous method. Any white turbidity in the filtrate during the washing may be ignored, since a number of tests have proved it to be free from tungsten.

Should a pellicle of tungstic acid adhere firmly to the beaker, it may be dissolved in a few ml. of ammonium hydroxide and re-precipitated by the addition of a little tannin, hydrochloric acid, filter-pulp and cinchonine solution. It is convenient to do so before the bulk of the precipitate is rinsed back into the beaker for washing. The precipitate is ignited wet in a tared porcelain crucible and weighed as tungstic oxide (WO_3).

SUMMARY.—The recovery of tungstic acid from sulphate solutions containing alkali sulphate, by precipitation with mineral acid or cinchonine, is shown to be incomplete. The tannin-cinchonine method of Schoeller and Jahn provides a rapid and accurate means of recovering tungstic acid from such solutions. A modification of the same method, by which more substantial amounts of tungstic acid (more than 0·2 g.) can be determined, has been worked out.

In conclusion, I desire to record my indebtedness to Dr. W. R. Schoeller for his kindly interest and helpful criticism.

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An Improved Universal Buffer

BY W. C. JOHNSON AND A. J. LINDSEY, PH.D., A.I.C.

UNIVERSAL buffer solutions have been described by the following authors: Prideaux,[1] Prideaux and Ward,[2] Michaelis,[3] and Britton and Robinson.[4] The most satisfactory of these was the mixture recommended by Britton and Robinson. This solution, 0·0286 molar with respect to hydrochloric acid, citric acid, potassium dihydrogen phosphate, veronal and boric acid, acted as a buffer between *p*H 2·6 and 12·0, and titration with 0·2 *N* sodium hydroxide solution gave a change of *p*H which was rectilinear between the values of 4 and 8. A solid mixture, containing no hydrochloric acid and having the advantage of portability and small storage space, was recommended to serve the same purpose. This mixture was dissolved and titrated either with sodium hydroxide for *p*H values over 3·91 or with hydrochloric acid for lower values.

We have effected a simplification by preparing a solid mixture, using free diethylbarbituric acid instead of the sodium salt employed by Britton and Robinson. It is thus possible to prepare buffer solutions of any *p*H between 2·6 and 12·0 by titrating a solution of this mixture with 0·2 *N* sodium hydroxide solution. Citric acid (6·008 g.), potassium dihydrogen phosphate (3·893 g.), boric acid (1·769 g.) and diethylbarbituric acid (5·266 g.) form the solid mixture which, when dissolved in water and made up to one litre, is ready for use. All the chemicals employed were of analytical reagent quality, with the exception of the diethylbarbituric acid which conformed with the B.P.C. standard.

The *p*H values of mixtures of 100 ml. of the solution with various quantities (x_1) of 0·2 *N* sodium hydroxide solution free from carbonate, were determined at

18° C. A hydrogen electrode[5] connected by means of a saturated salt bridge with a saturated calomel half-cell was employed for this purpose, and the potentials were measured with either a Cambridge or a Tinsley potentiometer. A further series of measurements (x_2) was made upon mixtures after dilution to a constant volume of 200 ml. As was expected, the results agreed well with those of Britton and Robinson, but results up to 0·1 *p*H lower were obtained between *p*H 7·5 and 8·5. Further, values a little higher than those of Britton and Robinson were obtained between *p*H 2·6 and 4·0. The linear equation, $p\text{H} = 0{\cdot}0853x + 2{\cdot}686$ holds accurately over the range from *p*H 4 to *p*H 7·5, and more approximately (within 0·1 *p*H) over the wider range to *p*H 9·0. For values outside this range quantities taken from the table below should be employed. The table showing even increments in *p*H was constructed by interpolation from the results of the titrations.

*p*H	x_1	x_2	*p*H	x	*p*H	x
2·6	2·0	0·0	5·8	36·5	9·0	72·7
2·8	4·3	2·4	6·0	38·9	9·2	74·0
3·0	6·4	5·0	6·2	41·2	9·4	75·9
3·2	8·3	7·3	6·4	43·5	9·6	77·6
3·4	10·1	9·3	6·6	46·0	9·8	79·3
3·6	11·8	11·1	6·8	48·3	10·0	80·8
3·8	13·7	12·9	7·0	50·6	10·2	82·0
4·0	15·5	14·1	7·2	52·9	10·4	82·9
4·2	17·6	16·9	7·4	55·8	10·6	83·9
4·4	19·9	19·6	7·6	58·6	10·8	84·9
4·6		22·4	7·8	61·7	11·0	86·0
4·8		24·8	8·0	63·7	11·2	87·7
5·0		27·1	8·2	65·6	11·4	89·7
5·2		29·5	8·4	67·5	11·6	92·0
5·4		31·8	8·6	69·3	11·8	95·0
5·6		34·2	8·8	71·0	12·0	99·6

In the first part of the table the two values of x recorded have the significance indicated above. For *p*H values above 4·4 x_1 and x_2 have almost identical magnitude. The quantities x_1 are particularly useful in the determination of *p*H by the following method of matching the hydrogen ion activity of an unknown solution.

The electrochemical cell:

Pt electrode	buffer and quinhydrone	salt bridge	unknown soln. and quinhydrone	Pt electrode

is set up, and the buffer solution is titrated with sodium hydroxide until there is no potential difference between the electrodes. A simple pointer galvanometer may be employed to indicate the null-point and no potentiometer is therefore required. The *p*H is found from the amount of sodium hydroxide used to produce a balance point.

This modified buffer solution has been used by us for several years. We have found it of considerable value in the rapid preparation of solutions of definite *p*H. It has the merit that only one standard solution need be kept and that the results are trustworthy to within a small fraction of a *p*H unit.

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Colour Tests for Chlorine, Ozone and Hypochlorites with Methane Base

BY A. T. MASTERMAN, M.A., D.Sc., F.R.S.

MOST of the colour tests for ozone aim at its identification in the gaseous state. The tests here described are based on the reactions of ozone and chlorine in solution, which can be compared with the corresponding reactions of hypochlorites.

Arnold and Mentzel[1] studied various tests that had been proposed for ozone (metaphenylenediamine, guaiacum, thallous salts, etc.), and found that they were not distinctive. They recommended the use of an alcoholic solution of 4.4′-tetramethyl-diamino-diphenylmethane ("methane base" or "tetramethyl base"). Test-paper soaked in this solution gives a blue colour with chlorine or bromine gas and a violet colour with ozone. Their results were confirmed by Fischer and Marx.[2] Even this reagent, however, has certain limitations, as is shown below. The fluorescein test (Benoist[3]) is sensitive, but hypochlorites (at all events when impure) also discharge the colour and the fluorescence. In the present investigation the behaviour of methane base with liquids containing chlorine hypochlorite and ozone respectively has been studied.

METHANE BASE.—The base is readily soluble in carbon tetrachloride, but less soluble in alcohol or acetone. If ozone or chlorine gas is led into such solutions a range of colours is produced. The presence of an acid appears to be necessary for the colour reactions; acetic acid is usually added, but equally good reactions, although slightly differing in the shade of colour, can be obtained with hydrochloric or phosphoric acid. The same range of colours is produced by leading ozone through dilute alcoholic solutions or by exposing the solutions to ultra-violet rays; the concentration of the solution affects only the intensity of the colours.

First an indigo-violet colour is produced, which gradually deepens in intensity to purple-violet or amethyst, and then gradually changes to rose, and finally, after some time, to reddish-amber or ruby-red. These "ozone colours" are probably stages in the progressive oxidation of the methane base, and their gradual formation, after supply of ozone is discontinued at the violet stage, is characteristic.

If chlorine gas is led through any of the solutions of methane base a blue colour is produced. In a carbon tetrachloride solution acidified with glacial acetic acid the blue changes gradually to green and finally to yellow. If dilute acetic acid is used the same series of colours is produced, but is confined to the supernatant aqueous layer. In no instance was it possible to obtain with chlorine gas any of the above-mentioned series of "oxygen colours" produced by ozone.* If water is added to the alcoholic methane base solution, the chlorine colours are much intensified and an extended series is produced. The deep (Solway) blue first formed changes successively to grass-green, olive-green, orange and yellow, and finally the solution becomes colourless. As the colours pass from blue through grass-green a transient crimson colour can also be seen by transmitted light, but this disappears when the olive-green stage is reached.

The reactions of methane base with ozone and with chlorine in solution may, therefore, be summarised as follows:

	Ozone		Chlorine
1	Violet	1	Blue
2	Amethyst	2	Grass-green
3	Rose	3	Olive-green
4	Ruby-red	4	Orange
	—	5	Yellow

HYPOCHLORITES AS CHLORINATORS.—Various types of hypochlorites (see Table I) were used. From the analyses in the table it will be seen that in E the hypochlorite was in the form of hypochlorous acid and no sodium salts were present. F had a low alkalinity, whilst C and D were more alkaline than A and B.

TABLE I

ANALYSES OF TYPICAL HYPOCHLORITE SOLUTIONS†

(Each adjusted to contain 5 g. of available chlorine per litre)

	Sodium hypochlorite g. per litre	Sodium chlorate g. per litre	Sodium chloride g. per litre	Sodium oxide g. per litre
A ..	5·25	0·599	86·6	0·34
B ..	5·25	1·146	54·0	0·42
C ..	5·25	0·427	6·88	0·96
D ..	5·25	0·33	5·26	1·00
F ..	5·25	0·141	4·07	0·19

	Hypochlorous acid g. per litre	Calcium chlorate g. per litre	Calcium chloride g. per litre
E ..	3·71	0·71	7·7

* Sometimes the chlorine yellow may assume an amber tint which is difficult to distinguish from the ruby-red "oxygen" colour.

† A, an electrolytic hypochlorite produced commercially in England; B, an electrolytic hypochlorite produced commercially in America; C, a chemically prepared hypochlorite, from bleaching powder and sodium carbonate; D, a similar product to which boric acid has been added (after Dakin); E, a solution of hypochlorous acid usually termed Eusol; F, a commercial hypochlorite, chemically prepared.

In aqueous solutions a hypochlorite produces with methane base a range of "chlorine" colours ending with complete bleaching. The following method of applying the test was finally adopted.

Three ml. of a 1 per cent. solution of methane base in 95 per cent. alcohol were placed in each of a series of test-tubes, and into each tube was introduced 40 ml. of dilute acetic acid (1·5 ml. of 5 *N* acid + 98·5 of water). Increasing doses of the hypochlorite (0·5 ml. to 10 ml. or more) were added to the acid reagent, and finally the contents of each tube were made up to 55 ml. with the dilute acid, well shaken, and left for several days. As a rule, when the hypochlorite was added prior to the dilution with the acid the colours were more brilliant. These colours are identical with those produced by chlorine gas and appear in the same order. Their formation depends strictly upon the relative quantity of hypochlorite to methane base, and by calculating a coefficient from $\frac{\text{mg. of methane base}}{\text{mg. of available Cl}}$, the following colour scale can be constructed:

6 to 1·5	.. ..	Blue, increasing in intensity
1·5 ,, 1·0	.. ..	Blue-green to grass-green
1·0 ,, 0·8	.. ..	Olive-green to brown
0·8 ,, 0·6	.. ..	Orange
0·6 ,, 0·5	.. ..	Yellow
0·5 ,, 0·3	.. ..	Primrose-yellow
0·3 ,, 0·0	.. ..	Colourless

The figures do not appear to be affected by the use of hypochlorite or methane base of different concentrations. The colours are somewhat transitory, especially the greens, olive-green and orange, and the series thus eventually becomes one of blues and pale yellows with a greyish intermediary.

Tests were made at time intervals to ascertain if the change of colour is due to the further chlorinating action of available chlorine in the solution, and the following results were obtained*:

Methane base / Available chlorine	Immediate	1 hour	2 to 8 hours	24 hours
3·0	—	—	—	—
2·0	—	—	—	—
1·7	+	—	—	—
1·5	+	—	—	—
1·36	+	+	—	—
1·20	+	+	+	—
1·10	+	+	+	—

In the immediate reaction available chlorine persisted in all the series except the two weakest.

After 1 hour all active chlorine was lost in the series down to 1·5, which covers all the blue colours. The transitory greens and oranges from 1·5 downwards are in a region where the chlorine persists for some hours, so that further changes in colour might be expected.

Titration of methane base against a hypochlorite (iodide and starch) shows that 2·5 mg. of the former are equivalent to 1 mg. of the latter. In any mixture

* + indicates the presence of available chlorine; — its absence.

in which the coefficient is less than 2·5/1, free active chlorine will be present. It appears therefore that the colours beyond blue (green, orange and yellow) are produced by further chlorination of the blue products first formed. It seems reasonable to assume that a blue and a yellow compound are formed and that other colours are produced by a mixture of the two.

At the end of 24 hours a period of comparative stability is reached (see Table II). All the series with a coefficient below 1·0 were completely bleached and those from 1·0 to 1·5 had lost their original tints of green and orange.

TABLE II

COLOUR REACTIONS OF CERTAIN HYPOCHLORITES WITH METHANE BASE IN AQUEOUS SOLUTION

(After 24 hours)

No.	Hypochlorite ml.	Available Cl mg.	E	F	D	A	B	A with added KOH
1	0·5	2·5	Light blue	Light blue	Blue	Pale blue	Pale blue	Pink
2	1·0	5·0	Blue	Blue	Light blue	Blue	Blue	Fawn
3	1·5	7·5	,,	,,	,, ,,	,,	,,	Beige
4	2·0	10·0	,,	,,	,, ,,	,,	,,	Pale brown
5	2·5	12·5	,,	,,	Very pale blue	Violet-blue	Violet-blue	Brown
6	3·0	15·0	Light blue	Light blue	Faint yellow	Violet-blue	Violet-blue	,,
7	3·5	17·5	,, ,,	Pale blue	Faint yellow	Blue-violet	Blue-violet*	,,
8	4·0	20·0	Very pale blue	,, ,,	Faint yellow	Violet	Violet	,,
9	4·5	22·5	Very pale blue	Faint yellow	Colourless	Amethyst	Amethyst	,,
10	5·0	25·0	Very pale blue	Faint yellow	,,	Rose	Rose*	,,
11	5·5	27·5	Colourless	Nearly colourless	,,	,,	,,	,,
12	6·0	30·0	,,	Colourless	,,	,,	,,	Orange

* The violet and rose are paler than in A.

The difference in the reaction of chemically and electrolytically prepared hypochlorites is very pronounced (see Table II); only after 4 or more days' standing can faint rose or violet tints be discerned in the former. When ozone was passed through the solutions of the electrolytic series (A and B, Table I) the earlier blues were unaffected, whilst the "oxygen" colours were intensified. Since the electrolytic products contained slightly more sodium chlorate and much more sodium chloride than the chemically prepared hypochlorites, samples of the latter were adjusted to contain the same amounts of chlorate and chloride as the former, and were then tested in the usual way. The addition of chlorate alone did not cause any material change except a tendency towards a slight brownish tint in two of the samples, but sodium chloride had an intensifying effect upon the colours; this was also noticeable with the colours produced by ozone gas, probably because ozone is more soluble in sodium chloride solution than in water.

The addition of a large excess of potassium hydroxide converts the colours into a series of secondary tints. Alkali, in the small degree in which it was present in the samples of hypochlorites tested, promotes bleaching, but does not affect the nature of the colours. In support of the view that the differences between the two classes of hypochlorites may be due to the presence of ozone in the electrolytic class, it may be pointed out that Schönbein (1840) demonstrated the presence of ozone in the gases evolved during the electrolysis of acidulated water and of salt solutions, and that Archibald and Wartenburg[4] showed that the solubility of ozone in neutral salt solutions is often greater than in water, and that such ozone solutions are more stable. Thus in the electrolysis of brine or magnesium chloride, conditions are present for the production and retention of ozone as well as of hypochlorites. It is suggested that, although chemically prepared hypochlorites may originally contain no ozone, yet ozone may be generated by secondary reactions and give the typical colour reactions. At all events, it is clear that some oxidising agent other than hypochlorite is present in electrolytic hypochlorites and is slowly developed in chemical hypochlorites. It is known that sodium hypochlorite decomposes, on standing, into chloride and chlorate, and according to Clarens,[5] a chlorite is produced as an intermediate product. Foerster and Dolch[6] found that at 50° C. hypochlorite is decomposed into chlorite and chloride, with liberation of oxygen, and that the chlorite is then converted into chlorate and chloride. According to Levi and Natta,[7] solutions of chlorites are not decomposed at 100° C. in the absence of a catalyst, even when hydrogen and oxygen are passed through them, but are decomposed in sunlight, with the production of ozone. Possibly, therefore, the colour reactions given by chemical hypochlorites with methane base may be due to the slow formation of chlorite, which then breaks up, yielding ozone as one of its decomposition products.

Allmand and Spinks[8] have shown that the action of light upon gaseous mixtures of chlorine and ozone results in the synthesis of ClO_3; under suitable conditions a reversal of this reaction might produce free ozone.

Hypochlorite solutions not only absorb ultra-violet rays, but, according to Mallet,[9] will emit them. It is therefore conceivable that the ultra-violet rays given off by the hypochlorite will gradually produce traces of ozone, which will react with methane base. With such an interpretation it is possible that the electrolytic hypochlorites differ from the "chemical" products in their more active production of ultra-violet rays, but the difference between the two classes is so marked that it seems more reasonable to assume that the electrolytic products contain ozone produced in the process of electrolysis.

TESTS WITH FLUORESCEIN.—Benoist[3] found that a solution of 1 part of fluorescein in a million is decolorised by a trace of ozone, and that 1 part in 10 millions loses its fluorescence but not its colour. On the other hand, chlorine, even when present in 3 times the amount of fluorescein, does not destroy even the fluorescence of a solution of 1 p.p.m. Egorov[10] also used a standard alkaline solution of fluorescein for the quantitative estimation of ozone, and the qualitative value of the test was confirmed by Allen.[11]

Standard aqueous solutions of fluorescein ranging from 1 to 10 p.p.m. were prepared. Five ml. of the hypochlorite solutions under examination were added

to 10 ml. of the 10 p.p.m. solution, the mixture was allowed to stand, and the rates of bleaching were determined from time to time by comparison with the standards. The results (Table III) showed that the chemically prepared hypochlorites had much less effect than the electrolytic products on the fluorescein. For example, in one series of tests, whilst the solution containing sample A was completely bleached at the end of two hours, D was not completely decolorised even after 5 hours. Sample E (Table I), having an acid reaction, was instantly decolorised, so that it was not available for this test.

TABLE III

REACTION OF CERTAIN HYPOCHLORITES WITH FLUORESCEIN

(Degrees of colour)

		Parts of fluorescein per million									
		10	9	8	7	6	5	4	3	2	1
Hours:		½	1	1½	2	2½	3	3½	4	4½	5
Samples	D	10	8	6	5/4	3/2	2/1	2/1	1	1/0	1/0
	F	10	7	6/5	5/4	3	2/1	2/1	1	1/0	0
	B	4/3	2	1	0	—	—	—	—	—	—
	A	3	2/1	1/0	0	—	—	—	—	—	—

The presence of ozone in the electrolytic hypochlorites would afford a reasonable explanation of these differences.

Samples of A and C were examined spectroscopically at the National Physical Laboratory. It was found that both were opaque to radiations of wave-length shorter than 0·4μ, and since the principal and most characteristic ozone bands are in the ultra-violet region in wave-lengths of less than 0·3μ, it was not possible by this means to detect ozone in the solutions.

HYPOCHLORITES AS OXIDISING AGENTS.—Since hypochlorites can act as either chlorinating or oxidising agents according to the particular substances, *e.g.* metals,

TABLE IV

COLOUR REACTIONS OF HYPOCHLORITES TO METHANE BASE IN PRESENCE OF EXCESS OF ALCOHOL

	Hypochlorite ml.	Methane base		
		10 ml.	3 ml.	1 ml.
1	0·5	Violet, amethyst rose, to ruby-red	Violet, amethyst rose, to ruby-red	Amber
2	1·0	,, ,,	,, ,,	Pale amber
3	1·5	,, ,,	,, ,,	Very pale amber
4	2·0	,, ,,	,, ,,	Colourless (clear)
5	2·5	,, ,,	,, ,,	,, ,,
6	3·0	,, ,,	,, ,,	,, ,,
7	3·5	,, ,,	Pale ,, ,,	,, (cloudy)
8	4·0	,, ,,	Very pale yellow	,, ,,
9	4·5	,, ,,	,, ,, ,,	,, ,,

on which they act, an attempt was made to reproduce the "oxygen" series of colours by using hypochlorite as an oxidising agent. It was found that when excess of ethyl alcohol is present, the "oxygen" colours are produced. A 1 per cent. alcoholic solution of methane base was treated with the hypochlorite solution (5 g. of available chlorine per litre), 0·1 ml. of 5 *N* acetic acid was added to each sample, and the mixture was made up to 55 ml. with alcohol. The results are shown in Table IV.

In every instance a blue colour was first formed, but disappeared on shaking, and then in the 10-ml. series a complete range of colours, corresponding exactly with that formed by ozone, was obtained (see Table V). In these tests no marked difference in the reactions were observed between the chemical and the electrolytic hypochlorites. The conditions under which hypochlorite acts as an oxidising agent towards methane base are: (1) the presence of excess of alcohol with a minimum quantity of water; (2) excess of methane base over hypochlorite.

TABLE V

COLOUR REACTIONS OF CERTAIN HYPOCHLORITES WITH METHANE BASE IN ALCOHOLIC AND AQUEOUS SOLUTIONS

(3·5 ml. of hypochlorite; 5 g. of available chlorine per litre; 3·0 ml. of 1 per cent. methane base solution + 0·1 ml. of 5 *N* acetic acid)

		Water	Alcohol	Chemically prepared sodium hypochlorite		Electrolytically prepared sodium hypochlorite	
				5 mins.	1 to 3 days	5 mins.	1 to 3 days
Cloudy	A	48	0	White	Pale blue	Blue	Blue
	B	44	4	,,	,, ,,	,,	,,
	C	40	8	Yellow	,, ,,	,,	,,
	D	36	12	Olive	Greenish blue	,,	,,
	E	32	16	Green	,, ,,	,,	,,
	F	28	20	Blue	,, ,,	,,	,,
	G	24	24	Indigo	Grey	,,	Violet
Clear	H	20	28	Violet	Yellow (br.)	Pale green	,,
	I	16	32	Amethyst	,, ,,	Violet	Amethyst
	J	12	36	,,	,, ,,	,,	,,
	K	8	40	Rose	,, ,,	Amethyst	Rose
	L	4	44	,,	,, ,,	Pale amethyst	Ruby-red
	M	0	48	,,	,, ,,	Rose	Pale ruby-red

If the proportion of the two reagents is kept constant but the proportions of water and alcohol varied (Table V), it is found that whilst the water is in excess a series of "chlorine" colours is produced. This is followed by a complete series of "oxygen" colours from violet to ruby as the amount of alcohol rises from 50 to 100 per cent. The water apparently throws the methane base out of solution and thereby reduces the proportion of this reagent to the hypochlorite.

Only when the proportion of alcohol exceeds 50 per cent. is all the methane base held in solution. Moreover, the reaction between alcohol and hypochlorite tends to reduce the quantity of the latter very rapidly. In the presence of methane base in addition, the destruction of available chlorine is still more rapid.

One minute after mixing the available chlorine can be traced in mixtures

containing up to 50 per cent. of alcohol, but after five minutes there is an absence of available chlorine throughout the whole series, except the first two.

CONCLUSIONS.—(1) The addition of ozone gas to a solution of methane base gives a sequence of characteristic colours, namely, violet, amethyst, rose, and ruby red. The addition of chlorine gas to a solution of the base gives another sequence of characteristic colours, namely, blue, grass-green, olive-green, orange and yellow with final complete bleaching.

(2) Hypochlorites, in aqueous solution, give, in the first instance, the "chlorine" sequence of colours. They can also be made to give the "ozone" sequence, probably by direct oxidation, by concentrating the methane base in alcoholic solution.

(3) In aqueous solutions, electrolytically prepared hypochlorites give the sequence of "chlorine" colours with the base and also the complete sequence of "oxygen" colours, which become recognisable a few hours after the "chlorine" colours.

(4) Chemically prepared hypochlorites in the same solutions give no such "oxygen" colour reactions. After prolonged standing, these hypochlorites are capable of giving partial "oxygen" colours. These colours can in some degree be intensified and their production accelerated by addition of sodium chloride.

(5) Electrolytically prepared hypochlorites are capable of decolorising solutions of fluorescein much more rapidly than those prepared chemically.

(6) It is suggested that these important differences in colour reactions between the electrolytically and chemically prepared hypochlorites may be due to the presence of ozone in the former.

I desire to acknowledge the valuable assistance of Mr. E. A. R. Bousfield, B.Sc., A.I.C., in preparing and carrying out the extensive series of experiments herein detailed.

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Notes

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

THE OCCURRENCE OF CYANOGENETIC SUBSTANCES IN EDIBLE MEMBERS OF THE CRUCIFERAE

INVESTIGATORS of cyanogenetic substances often entirely ignore the presence of these compounds in members of the *Cruciferae* that are used as human foodstuffs. In view of this I am anxious to record such literature as I have collected and such experiments as I have had time to perform.

The earliest reference is by Hoffman,[1] who by steam-distillation of cabbages and other members of the sub-family *Brassica* obtained oils which, on further fractionation, gave nitriles. Phenyl propionic nitrile was found in watercress and phenyl acetic nitrile in nasturtium.

Further observations were not made until 1928, when Chesney *et al.*[2] found that prolonged feeding of cabbage to rabbits produced goitre. This is not surprising, since a well-known method of biological standardisation of thyroid preparations is by feeding mice with aceto-nitrile and the thyroid preparation whose value is to be assessed.

Marine, Cipra and co-workers[3] ascribed the following properties to the goitrogenic principle. Prolonged steaming of cabbage may actually increase the goitrogenic properties; drying by air or *in vacuo* causes loss of the agent; alkaline hydrolysis extracts or destroys it, but acid hydrolysis is much milder in its effect. The active agent is soluble in ether and ethereal solvents, and its amount varies with seasonal and climatic changes. These properties are easily explained by the known chemical behaviour of nitriles.

Later[4] the same workers showed that cyanides are goitrogenic by depression of oxygen capacity, and also that methyl cyanide produces exophthalmic goitre.

Cyanides are used in the estimation of circulatory rate, since they depress the activity of the carotid body and hence affect the respiratory rhythm, and in an investigation it was found that the urine passed in 3½ hours by persons who had previously partaken of cabbage and brussels sprouts at the immediately previous meal, contained from 1·6 to 8·6 mg. of sodium cyanide.[5] Prior to the meal the urines were free from cyanide.

G. V. JAMES

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THE BERNHARD BARON RESEARCH LABORATORIES
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May, 1939

A CHARACTERISTIC REACTION OF DITHIO-OXAMIDE WITH FERROUS IRON

DITHIO-OXAMIDE, rubeanic acid ($H_2N.CS.CS.NH_2$), is used as a very sensitive reagent for nickel, cobalt and copper (P. and R. M. Rây[1]). The reagent also gives reactions with a number of other metallic salts,[2,3,4] and with ferro-aquo ions ($Fe(CN)_5H_2O$),''' giving a blue colour.[5] With regard to its behaviour with iron salts, the relevant statements found in the literature are, that it does not give any precipitate with them,[6] and that iron does not interfere with the detection of copper or nickel.[7] On testing the behaviour of an alcoholic solution of dithio-oxamide with ferrous and ferric salts in neutral and acid solution, however, I obtained a reaction in which a dark precipitate, probably a ferrous salt of dithio-oxamide, was produced. This occurred when the reagent was mixed with a solution of ferrous salt (ferrous ammonium sulphate or ferrous chloride) containing sodium acetate. No further investigation of this reaction was made, because it did not seem to be distinctive. In alkaline solution, however, there was a distinct blue colour reaction, the characteristics of which were studied by three different methods:

(1) *Interaction of excess of cold alkaline solution of dithio-oxamide with a ferrous salt.*—When 1 or 2 drops of a 0·05 *M* neutral or slightly acid solution of ferrous salt were treated in a test-tube with 0·5 to 1 ml. of a freshly-prepared, cold alkaline solution of dithio-oxamide a deep blue colour, which changed on standing, was produced. The reaction was carried out with ferrous ammonium sulphate and with ferrous chloride, and the alkali used was sodium hydroxide in concentrations of 1·2 and 2·5 *N*. The concentration of the dithio-oxamide solution was 20 to 40 mg. in 20 ml. of alkali solution.

(2) *Interaction of excess of alkaline solution of dithio-oxamide with ferric salts in presence of a reducing agent.*—One or two ml. of an alkaline solution of dithio-oxamide (strength as above) were treated first with a little sodium hydrosulphite, $Na_2S_2O_4$, and then with 1 or 2 drops of a 0·01 *M* solution of a ferric salt (ferric ammonium sulphate or ferric chloride). The blue colour appeared immediately; even with a 0·001 *M* solution the reaction was distinct, though faint.

(3) *Solution of metallic iron in an alkaline solution of dithio-oxamide.*—It was found that metallic iron behaves in an interesting manner in an alkaline solution of dithio-oxamide. When a piece of electrolytic iron wire was left in the solution, the pale yellow colour showed very little, if any, change, even after 20 to 30 minutes; but immediately after the iron wire had been made cathodic for a few seconds in the solution against another iron wire as anode, or after it had been touched with a piece of zinc or aluminium wire, it began to dissolve, imparting an intensely blue colour to the liquid.

These facts seem to indicate that the colour is due to a reaction between bivalent iron and dithio-oxamide in alkaline solution. As found by P. and R. M. Rây,[1] nickel and cobalt also give *soluble* compounds with dithio-oxamide in presence of alkali. The blue ferrous compound, the colour of which is similar to that of the ferrous compounds of *iso*nitrosoketones, is not very stable. After some time it changes, probably owing to oxidation or decomposition.

GUSTAV NILSSON

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NOBEL INSTITUTE
STOCKHOLM, 50

May, 1939

A SIMPLE MICRO-TEST FOR COPPER

DURING an investigation on the reduction of evaporation from water surfaces by uni- and multi-molecular surface films, it was found that a drop of oleic acid, placed on a water surface, assumed a blue-green colour when certain samples of distilled water, which had been kept in a copper container, were used. A micro-test using sodium diethyl-dithiocarbamate,[1] revealed that the copper concentration was of the order of 5 in 10^5. On pursuing the matter further, it became evident that the blue coloration of oleic acid, which is due to the formation of copper oleate, may be used as a test for small amounts of copper. The colour is almost a pure blue when pure oleic acid is employed; with commercial samples of oleic acid the colour is blue-green or green owing to the yellow colour of the acid.

The test is almost as sensitive as that with sodium diethyldithiocarbamate, as is shown in Table I.

TABLE I

Concentration of copper	Oleic acid (0·05 ml. on 100 ml. of soln.)	Diethyl-dithiocarbamate
1 part in 10^4	Deep blue-green (green solid deposit after 1 day)	—
1 ,, ,, 10^5	Less deeply coloured	Turbid brown (prec.)
1 ,, ,, 10^6	Pale blue-green	Brownish-yellow
5 ,, ,, 10^7	Very pale	Pale lemon yellow
1 ,, ,, 10^7	No colour	Hardly perceptible yellow

Table I shows that a concentration of copper as low as 0·5 to 1 part in 10^6 can be detected when 100 ml. of solution and a drop of oleic acid of about 0·05 ml. are used. The sensitivity may be further increased by employing a larger volume of solution, since the method is virtually a concentration, in a small drop of oleic acid, of a large fraction of the copper present. In an experiment in which 1 litre of solution was used, the pale green colour was detected when the concentration of copper was only 1 in 10^7.

The *p*H of the solution should be between 5 and 8 (Table II). Within this range the intensity of the colour is practically independent of the *p*H. At *p*H values lower than 3 to 4 and higher than 9 to 10 no colour is observed. Copper oleate is obviously not very stable in presence of acid; on the other hand, in alkaline solutions the concentration of Cu^{++} is very small, owing to the formation of hydroxide. It is seen from Table II that the optimum range is between 5 and 7.

TABLE II

Concentration of copper	Colours at varying *p*H					
	3	4·3	5	6–7	8	10
1 part in 10^4	Nil	Deep blue-green	Deep blue-green			
1 ,, ,, 10^6	Nil	Nil	Pale green	Pale blue-green		Faint blue

When the drop of oleic acid is placed on the solution and the system is merely allowed to stand, the colour may take one day to develop. The time is considerably shortened, however, by stirring the solution continuously or simply by boiling. With a solution containing 1 part of copper in 10^6 the maximum colour appeared after 20 minutes on stirring and after 5 minutes on boiling (Table III).

It is advisable to stir the solution in such a way that no appreciable emulsification takes place, since the droplets may not unite readily after agitation has ceased. For this reason shaking is not advisable.

The test is not seriously affected by other metals which may be present in the

solution. Cobalt, nickel and chromium do not produce any colour with oleic acid, nor does manganese unless present in very large amounts. Ferric salts cause a reddish-brown coloration of the oleic acid due to the formation of ferric oleate. If the iron concentration is less than about 1 part in 10^4 no precautions need be taken, since the colour due to the iron is then only yellow, which turns the copper colour to green-yellow. If iron is present in larger quantities, its colour will interfere with the colour produced by the copper. In that event tartaric acid neutralised with sodium hydroxide should be added. To 100 ml. of $N/100$ ferric chloride solution, which contained tartaric acid that had been neutralised with sodium hydroxide (litmus), 1 ml. of water containing 1 part of copper in 10^4 was added, so that the end-concentration of copper was 1 in 10^6. With this solution the oleic acid test was carried out. The drop showed a definite pale blue-green colour which was easily visible above the yellow complex ferric salt solution.

TABLE III

0·05 ml. of oleic acid on 100 ml. of solution

Concentration of copper	Time of maximum colour on		
	Standing	Stirring	Boiling
1 part in 10^4	18 hours	20 mins.	Colour appears almost immediately
1 ,, ,, 10^5	18 ,,	20 ,,	
1 ,, ,, 10^6	2 days	20 ,,	5 mins.
5 parts in 10^7		½ to 1 hour	

Marcille[2] reports that the green colour occasionally exhibited by olive oils is due to a compound of copper with chlorophyll, which is sometimes present in the olive oil. Another possibility is that the colour is due to the formation of copper oleate. A drop of triolein placed on water which contained 5 parts of copper in 10^5, developed a green colour, obviously due to a certain amount of hydrolysis of the triolein.

E. HEYMAN
LUCY F. KERLEY

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CHEMISTRY DEPARTMENT
UNIVERSITY OF MELBOURNE

Official Appointment

THE Minister of Health has approved the following appointment:

DONALD CLARENCE GARRATT as Public Analyst for the Metropolitan Borough of Camberwell, in place of Martin Priest (retired).

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.

ROYAL BOROUGH OF KENSINGTON

Report of the Public Analyst for the Fourth Quarter, 1938

Of the 332 samples of food and drugs examined, 216 were purchased formally.

Hair Lotion containing Carbon Tetrachloride.—A bottle of hair lotion was submitted for analysis with the information that it had caused unconsciousness in a woman who had used it. It was found to consist essentially of a mixture of paraffin hydrocarbons with carbon tetrachloride. As the public cannot be expected to be aware of the anaesthetic and toxic properties of carbon tetrachloride, it seems desirable either that its sale in shampoos and preparations of a similar nature should be prohibited, or that it should be permitted only when labelled with a caution as to its dangers. F. W. Edwards

Legal Notes

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

PROCESSED CHEESE

In September of last year a firm was prosecuted and convicted at Slough for selling as "Finest Cheddar" a processed cheese which had been made from partly skimmed milk instead of from whole milk as is customary with hard cheeses of the Cheddar type. Since then the question of the composition of processed cheese has been taken further by a request from this Society to the Ministry of Health that a limit of water be fixed at a maximum of 42 per cent., with a minimum fat-content of 45 per cent. calculated on the dry matter.

The water-content of processed cheese has, in recent years, steadily increased and is still increasing, and, in an endeavour to put some check on the practice, a firm of processed cheese manufacturers was summoned, on May 26th, at Slough, under Section 30 of the Food and Drugs (Adulteration) Act, for selling cheese falsely labelled as "Gruyère" and "Packet Cheddar." The purchased sample, which bore the label "Gruyère," was found to contain 49·01 per cent. of water, and that labelled "Packet Cheddar," 48·27 per cent. of water. The prosecution maintained that, whilst Gruyère and Cheddar were essentially whole-milk cheeses containing, before processing, about 35 per cent. of water, there was no requirement in the process of manufacture that afforded any justification for the addition of such large extra quantities of water in making a satisfactory processed cheese. Evidence in support of this view was given by Mr. Eric Voelcker, F.I.C., the County Analyst, and by Dr. H. E. Cox, F.I.C. The amounts of water found were considered to be excessive and to correspond to the adulteration, with water, of milk, whiskey, or other food or drink. Since notice of the adulteration was not given, it was considered to be a fraud on the public.

For the defence Dr. E. J. Parry, F.I.C., contended that it was impossible to prepare cheese for packing in small sections without adding water, and he considered that the labels correctly described the cheeses.

The firm was convicted and fined £10 with 10 guineas costs. The Bench, when asked to fix a standard of water-content of packet cheese which could bear these labels, decided that 40 per cent. was a very fair standard.

It was stated in court that no further proceedings would be taken, up to September 30th, on stocks already sold to shops prior to the cases just heard.

There have recently been three further prosecutions. In the first of these a firm was fined £2 2s. 0d., with £6 6s. 0d. costs, at Henley-on-Thames, Oxfordshire, for selling Cheddar cheese containing 50·25 per cent. of water. The bench were not prepared to make any standard for water in cheese, but were willing to accept that laid down by the Slough magistrates. In the second case, also tried at Henley, on June 16th, a fine of £5, with £6 6s. 0d. costs, was imposed for the sale of Cheddar cheese containing 48·51 per cent. of water. The defendants in the third case pleaded guilty to selling "Finest Cheddar" cheese containing 51·44 per cent. of water, and on June 23rd were fined £10 0s. 0d., with £7 0s. 6d. costs, at Ivinghoe, Buckinghamshire.

Ministry of Health

THE BACTERIOLOGICAL EXAMINATION OF WATER SUPPLIES

The first report on this subject was published by the Ministry of Health in 1934 and reprinted in 1936. It has been revised and extensively re-written by an Office Committee, the names of the members of which are given below.† The Report is divided into two parts—General and Technical—and contains four appendixes, dealing with (A) Sampling, (B) Apparatus, (C) Media, and (D) Probability Tables.

Part I, General (pages 1–17) is divided into seven sections under the following headings: (I) Introduction, (II) Taking of Samples, (III) The Rationale of the Bacteriological Analysis of Water, (IV) Frequency and Type of Examination, (V) Interpretation of Results, and (VI) Suggested Classification of Waters.

Part II, Technical (pages 18–43), is divided into five sections: (VII) The Plate Count, (VIII) The Presumptive Coliform Count, (IX) The Differential Coliform Test, (X) The Faecal Streptococci Test, and (XI) The Clostridium Welchii Test.

The first part, although addressed to the non-technical reader, will be found of value by medical officers of health, water engineers and sanitary inspectors. It includes a simple description of the rationale of bacteriological examinations, considers the frequency and type of the examinations necessary, discusses at some length the interpretation of results, suggests a classification of piped water supplies into classes described as Highly Satisfactory, Satisfactory, Suspicious and Unsatisfactory, and gives appropriate standards for small rural supplies such as shallow wells, based on the Presumptive Coliform Count. Attention is drawn to the advantage of examining a number of samples by a simple test rather than occasional samples by more complicated tests, and it is suggested that a simple

* Reports on Public Health and Medical Subjects, No. 71 (Revised Edition). H.M. Stationery Office, 1939. Price 1s. net.

† Ministry of Health Memo. No. 221. T. Carnwath, D.S.O., D.Sc., M.B., D.P.H. (Ministry of Health) (Chairman); J. F. Beale, M.A., M.R.C.S., L.R.C.P., D.P.H. (Counties Public Health Laboratories); D. B. Byles, B.Sc., F.I.C. (Deputy Director of Water Examination, Metropolitan Water Board); H. T. Calvert, M.B.E., D.Sc., F.I.C.; W. M. Scott, M.D., B.Sc., D.T.M. & Hy., and E. L. Sturdee, O.B.E., M.R.C.S., L.R.C.P., D.P.H. (Ministry of Health); E. W. Taylor, M.A., M.D., D.P.H. (Senior and Research Bacteriological Assistant, Metropolitan Water Board); Professor G. S. Wilson, M.D., F.R.C.P., D.P.H. (Professor of Bacteriology, London School of Hygiene and Tropical Medicine).

Coliform examination should be carried out daily by the larger water undertakings, and as frequently as possible by the smaller ones; at the same time the lesser importance of very frequent examination for upland surface supplies derived from moorland where there is little possibility of human contamination is admitted, and the greater importance of frequent examinations of water from deep wells sunk in fissured strata is emphasised.

In the second part of the Report the technique of the Coliform Count is dealt with at some length as being of major importance. What is known as the sampling error of the liquid dilution method (*i.e.* the error due to the chances of catching or missing a single bacterium in the particular quantity of sample taken for cultivation from the critical dilution) is considered, and the best way of eliminating this element of chance is described. This is effected by taking several equal portions and calculating the numbers present from tables compiled from the laws of probability by McCrady and modified by Swaroop. Such tables are given in an Appendix.

Four methods for the differentiation of coliform bacilli are given. In the first method (A) primary cultures are made in MacConkey's broth at 37° C., and plated out on bile salt agar after 24 hours, and colonies are selected for the M.R., V.P., citrate, indole and gelatin-liquefaction tests. In the second method (B) the cultures in MacConkey's broth are incubated at 37° C., and those showing gas formation are subcultured in MacConkey's broth at 44° C. Gas formation at the latter temperature indicates Type I or Type II (faecal) *B. coli*. If no gas is shown at 44° C., the tubes showing gas at 37° C. are subcultured in Koser's citrate broth and incubated at 37° C.; growth in 2 to 3 days is regarded as presumptive evidence of organisms of the Intermediate-Aerogenes-Cloacae type. In the third method (B_2) the procedure is the same as in method (B_1), but if the MacConkey tubes incubated at 44° C. show no gas, those incubated at 37° C. are plated out, and colonies are selected and identified. In the fourth method (B_3) the primary cultures in MacConkey's broth are incubated at 44° C. Acid and gas production indicates faecal *B. coli*, and no account is taken of the other types. It is possible that the faecal *B. coli* content may be slightly underestimated by this method. The importance of maintaining the temperature strictly at 44° C. is emphasised. It is suggested that the simpler means of differentiation should be given a trial.

D. R. W.

Department of Scientific and Industrial Research

WATER POLLUTION RESEARCH BOARD

Report for the Year ended June 30th, 1938

This, the Eleventh Annual Report of the Board, includes the Report of the Director (Dr. H. T. Calvert). The Report once more emphasises the necessity for constant vigilance on the part of those responsible for the supply of water to the public. The serious outbreak of typhoid fever at Croydon which, it is pointed out, was due to the infection of part of the public water supply of the district, focussed public attention on the importance of taking all practicable steps to protect sources of water from pollution, of systematic treatment of all supplies in any way liable to pollution, and of frequent examination of samples to ensure that the treatment is adequate. The Board say that any neglect of the lessons to be learnt from the Croydon outbreak would be deplorable. The research undertaken under the supervision of the Board includes investigations on the treatment of water for public supply and for industrial purposes, the treatment and disposal of domestic sewage and industrial effluents, and problems of pollution of rivers.

TREATMENT OF WATER WITH SYNTHETIC RESINS.—The discovery (*cf.* ANALYST, 1936, **61**, 33; 1937, **62**, 302; 1938, **63**, 430) in the course of the Board's work that acids, bases and salts can be removed from solution in water by means of certain synthetic resins has aroused widespread interest, both in this country and abroad, and considerable progress has been made in the development of the resins for industrial purposes by commercial firms who have acquired licenses for their manufacture and sale. The main applications of the resins up to the present have included the treatment of water to remove all or part of the dissolved salts; this had previously been possible only by more expensive methods, such as distillation. Utilisation of the resins for the removal of valuable substances, such as metals, from very dilute solutions (for example, from industrial effluents), and for the removal of objectionable substances in very low concentration in water is also being developed rapidly. Recent work under the Board has shown that, under certain conditions, fluorides, which are sometimes present in water in low concentrations, may be removed by *m*-phenylenediamine resin; the fluorine is not readily removed, however, when other salts usually found in natural waters are present. An important point in connection with the use of these resins for the treatment of drinking water is that, when freshly prepared, they contain some organic matter which is soluble in water. This soluble matter can, however, be removed from some of the resins by extraction several times with hot water.

LEAD IN DRINKING WATER.—The investigation to determine the average quantity of lead taken up by certain types of water from lead pipes and fittings under conditions of household supply has been continued, and tests have now been made on seventeen services in different parts of England and Scotland. The extent to which the water is contaminated by passage through lead pipes depends on the character of the water, the length and arrangement of the pipes, the rate of flow of water and other conditions. In consequence, large differences may occur from time to time in the concentration of lead in water withdrawn from any one service. In one of the tests, extending over several weeks, the average concentration of lead in the water was as high as 0·5 part per million; this concentration is too high if risk of lead poisoning is to be avoided. Experiments are in progress on methods of treatment of waters with the object of reducing their action on mains and service pipes of different materials. These methods, in general, aim at reducing the acidity or increasing the alkalinity of the water. At some works alkalis, such as lime, soda and calcium carbonate are added, in controlled quantities, to the water; at other works the water is passed through filters containing pieces of limestone, marble or magnesite (*cf.* ANALYST, 1938, **63**, 302).

STERILISATION OF SLOW SAND FILTERS.—For many years at a water works in Scotland slow sand filters had been used, and the filtered water had been of high quality. After the periods of drought in 1933 and 1934 an additional storage reservoir was brought into service, and six weeks later *B. coli* were found in 1 ml. of the filtered water. The primary and secondary slow sand filters were then sterilised with sodium hypochlorite, and within six days the high quality of the filtered water was regained, no *B. coli* being found in 100 ml. The method of treatment is outlined.

MILK FACTORY EFFLUENTS.—One of the most important of the Board's investigations is the work which is being carried out, in collaboration with the milk industry, on the treatment and disposal of waste waters from dairies and milk products factories. These waste waters have in the past caused serious pollution of streams into which they have been discharged. In 1935, after preliminary laboratory experiments, the Board erected two large-scale experimental plants at a milk-collecting and distributing depot and cheese factory at Ellesmere, Shropshire. Previous Annual Reports (*cf.* ANALYST, 1937, **62**, 431; 1938, **63**, 302) have described the treatment of milk washings from the milk depot. It was shown that these liquids, which are difficult to treat by the method of biological filtration

as ordinarily applied at sewage disposal works, could be satisfactorily purified when passed at a controlled rate through two percolating filters in series; the order in series is reversed periodically in order to prevent the accumulation of excessive amounts of solid matter in the filters. During the past year the waste waters treated have contained whey washings, which are produced during the manufacture of cheese. These washings are rather more difficult to purify than milk washings, but effluents of excellent quality have been obtained. With two percolating filters in series, the quantity of polluting matter which can be treated daily in filters of a given size is considerably greater than is usual in the treatment of sewage at a sewage disposal works, where the liquid is passed through only one filter. The new method has been adopted and is in satisfactory operation at a number of milk depots and cheese and butter factories. During the period 1935 to 1938 the industry contributed approximately £10,000 towards the cost of the investigation.

TREATMENT OF TOWN SEWAGE.—The new method of operating percolating filters has also aroused great interest among those concerned with the treatment and disposal of town sewage. The Birmingham, Tame and Rea District Drainage Board, which uses filters of a capital cost of nearly £1,000,000, has placed a new laboratory and suitable large-scale plant at the disposal of the Board in order that treatment of sewage by the new method may be tried on a large scale. The whole of the cost of the new laboratory and of necessary additions and modifications to the plant has been met by the Drainage Board.

ACTIVATED SLUDGE PROCESS OF SEWAGE TREATMENT.—The investigation, under the direction of Professor J. C. Drummond, has been continued (*cf.* ANALYST, 1937, **62**, 432). The results of experiments, described in detail, indicate that the disappearance of nitrite during the aeration of unsterilised mixtures of sewage and activated sludge is due to biological activity and not to simple chemical decomposition.

COLLOIDS IN SEWAGE.—Further work, under the supervision of Professor F. G. Donnan (*cf.* ANALYST, 1937, **62**, 432), has shown that change in the area of the surface of the bubbles, when gases were passed through sewage and through mixtures of sewage and activated sludge under the conditions of the experiments, had no appreciable effect on the amount of organic matter flocculated. Temperature has been found to have a pronounced influence on the coagulation, during aeration, of organic matter in mixtures of sewage and activated sludge. Aeration of sewage treated with synthetic resin (*m*-phenylenediamine resin) caused a comparatively rapid removal of organic carbon from the supernatant liquid, but the addition of this resin had no marked effect on the rate of removal of total nitrogen. On the other hand, the addition of sulphited quebracho tannin resin increased the amount of total nitrogen removed from sewage by aeration and centrifuging, but retarded the rate of removal of organic carbon; there was in fact an initial rise in the concentration of organic carbon. The results of experiments on the effect of proteins and various organic materials, together with sodium chloride, on the rate of sedimentation of quartz particles at various *p*H values are also described in detail.

RIVER MERSEY INVESTIGATION.—The investigation on the River Mersey, which was begun in 1933 and occupied about 4 years, has been completed and a comprehensive special report has been published as Water Pollution Research Technical Paper No. 7. A full summary of the experiments, observations and conclusions is included in the present Report. The work was undertaken at the request of the Merseyside Local Authorities, the Mersey Docks and Harbour Board, and other local interests, who agreed to meet the whole of the cost. Its object was to determine the effect of the discharge of crude sewage into the Estuary on the amount and hardness of the estuarine deposits. This problem, which was obviously one of considerable importance to the authorities responsible for the

conservancy of the river and for the disposal of sewage of the Merseyside towns, had for many years been the subject of much local controversy. The investigation, which cost about £26,000, has been eminently successful in that it has given a definite answer to the difficult question asked in the terms of reference. It has led to the conclusion that the crude sewage discharged into the Estuary of the River Mersey has no appreciable effect on the amount and hardness of the deposits in the Estuary.

ATMOSPHERIC POLLUTION

Nineteen representatives of local authorities and other organisations co-operating with the Department of Scientific and Industrial Research in investigations into the nature and extent of atmospheric pollution met on June 6th in half-yearly conference at the offices of the Department. The gathering included representatives from Birmingham, Cardiff, Dagenham, Glasgow, Halifax, Leeds, Liverpool, London County Council, Manchester, Newcastle, Rotherham, Sheffield, Scunthorpe, Stoke-on-Trent, Willesden, Leicester, British Commercial Gas Association, and British Electrical Development Association.

Alderman D. Adams, M.P., J.P., of Newcastle, presided, and, at the close of the meeting, was unanimously re-elected Chairman of the Conference for a third year.

The Conference learned with interest of measurements which had been made in Scunthorpe by Dr. W. H. Hartston (the Medical Officer of Health), on the amounts of zinc and lead in the matter deposited from the air. Other members of the Conference who had had previous experience of measurements of zinc, lead and other metals in the atmosphere of steel-manufacturing and other towns, also took part in the discussion.

The Conference expressed its satisfaction at the welcome which had been given to the Public Health (Coal Mine Refuse) Bill in its recent passage through the House of Commons.

Dr. G. M. B. Dobson, F.R.S. (Chairman of the Atmospheric Pollution Research Committee) presented a report on the progress of the investigations carried out under the Committee. He told the Conference that the special survey of atmospheric pollution in and around the City of Leicester had now entered upon its third year, during which special attention would be paid to certain problems which the previous work of the survey had shown to be important. This had required the establishment of three new observing stations, for the measurement of dust in the air, near the city. Special work on these problems would also be carried on in the Laboratory during the year.

Royal Agricultural Society of England

ANNUAL REPORT OF THE CONSULTING CHEMIST

THE Report of the Consulting Chemist (Mr. Eric Voelcker, F.I.C.) gives *inter alia* particulars of matters of interest arising out of work done in the Society's Laboratory during the year. A striking feature was the number (43 out of a total of 127) of samples of water sent for analysis. This is a large increase and may have been due to the recent water scare, but several samples were went by members proposing to purchase new property.

HOUSEHOLD REFUSE AS SOURCE OF HUMUS.—This material is prepared from household refuse from which the bulk of the cinder and clinker has been removed by screening, after which the glass, tin, crockery, rags and some of the paper are picked out by hand. The particles of these that get past the pickers will be very small and not of any consequence. So far as possible the product, which is non-odorous, consists of the organic matter in the original refuse. Preliminary results of field trials indicate that it is of about the same value as dung, and with the increasing difficulty of obtaining sufficient farmyard manure, it may become of great importance not only to farming, but also to market garden production and horticulture.

Composition of "Horse Gram."—This is an Indian legume (*Dolichos biflorus*) which is largely used in India as cattle food, and the crop is grown both for fodder and for the seed. A sample gave the following analytical results:—Moisture, 9·57; oil, 0·43; albuminoids 22·87; carbohydrates, etc., 58·63; woody fibre, 4·93; mineral matter (ash), 3·57 per cent. The nitrogen-content was 3·66 per cent., and the ash included 0·60 per cent. of sand and silica. This material is quite distinct from the common gram or chick pea (*Cicer arietinum*). At times it has been imported into this country, but apparently not of recent years. It was found free from cyanogenetic glycosides and would appear to be a suitable seed for grinding and adding to animal foods. If it could be delivered here at a cheaper rate than peas or beans it would be worth a trial, but not otherwise.

Government of India

REPORT ON THE CHEMICAL LABORATORIES MAINTAINED BY THE CENTRAL BOARD OF REVENUE FOR 1937–38*

THE Control Laboratory (under the direction of the Special Chemical Adviser, Dr. H. B. Dunnicliff) remains in the Chemistry Department of the Government College, Lahore, but the plans and estimates for the building and equipment of the Control Laboratory at New Delhi have been finally approved. The laboratories of the Board render services to other Departments of the Government of India. Thus, all the Customs laboratories have been supplied with the standard apparatus for testing petroleum, and arrangements have been made whereby imported explosives shall be tested in those laboratories.

CLASSIFICATION OF SPIRITUOUS, MEDICINAL AND TOILET PREPARATIONS.—At the Excise Conference, held at Delhi in November, 1937, the question of the uniformity in rate of duty on spirituous medicinal and toilet preparations was discussed, and it was found necessary to divide them into three classes: (1) medicinal preparations of undeniable therapeutic value; (ii) preparations which,

* Published by the Manager of Publications, Government of India Press, New Delhi, 1939. Price 1s. 3d.

although commonly used in dispensing, were more of the nature of flavouring agents than medicines (*e.g.* Sir. Aurantii), or which could be used as beverages (*e.g.* tonic or medicated wines); (iii) toilet preparations and perfumery. It was decided that lists of preparations falling in class (ii) should be prepared by a technical co-ordinating officer appointed for all the Provincial Governments, and that this officer might most appropriately be under the control of the Central Board of Revenue.

INTERPRETATION OF THE TERM "DYEING."—The exact meaning of the terms "dyes" and "dyeing process" has again assumed some importance. The Board ordered that the term "dyeing" should be interpreted to mean the imparting of colour to textiles, leather, paper, fur and similar articles. The new definition necessitated the revision of the "Standard List of Tested Dyes" issued by the co-ordinating officer, the Chemical Examiner, Bombay Custom House. It was formerly the practice to consider coal-tar colours used in varnishes, soaps, etc., also as dyes under Sec. 30(1) of the Indian Customs Tariff.

DEFINITION OF GREASE.—The Board held that, for purposes of assessment, the term "grease" should be deemed to refer to substances which are solid at ordinary temperature. In cases of doubt the question whether an article should be classified as a "grease" or an "oil" should depend on the result of the "pour-point" test, whether or not the preparation contained soap.

Proposals on what should be considered as "lubricating greases," as distinguished from lubricating oils as specified in the Tariff, and in which cases and to what extent laboratory tests should be made have been adopted for circulation to the ports for opinion.

MORPHINE-CONTENT OF OPIUM.—The questions of the maximum morphine-content of Indian opium and the loss of morphine from opium on storage are receiving attention. The highest morphine strengths recorded in samples of opium collected from first lancings were as follows:

Opium from	Morphine, by B.P. method Per Cent.
Ghazipur	18·3
Gorakhpur	18·1
Gonda	17·2
Bareilly	16·8
Shahjehanpur	17·0

The remnants of these samples have been sealed in bottles for examination after some time, to ascertain if any fall in morphine strength occurs.

DEGRADATION OF OPIUM.—The degradation of opium in Malkhana vats, first observed in 1935, has caused some anxiety, and a systematic investigation to discover the cause and prevention of the trouble is in progress. In particular, an attempt is being made to determine if pasewa associated with opium during the time of collection has any connection with this deterioration. Although the existence of pasewa has been known for more than sixty years (see John Scott, *Report on the Experimental Cultivation of Opium Poppy*, Season 1876–77), this important subject has never been properly studied. The exact nature of pasewa is unknown, and no recent work has been done on its development or composition, with the exception that volatile aliphatic acids, especially acetic acid, have been detected in it. Preliminary work on cultivators' samples of doubtful origin has been begun, and a full investigation will be undertaken when authenticated samples collected at the time of the next opium collection (1939) have been received from the Opium Agent.

Ceylon

REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1938

THE Government Analysts' Department (under the direction of Mr. J. V. Collins) now includes 5 chemists and 2 probationers, all of whom are qualified to sign reports and accept responsibility for their work. Prior to 1938, reports were signed only by the two senior members of the staff, who had thus to accept responsibility for analyses not actually made by them—obviously an improper arrangement if any of the reports had to be defended in court.

The amount of work submitted to the Department continues to increase. In 1938, 4095 articles were examined as compared with 3684 in 1937. In the 6 years since 1933 the number of reports issued has almost doubled. A similar increase has occurred in the number of criminal cases requiring investigation. The policy of sending police officers to be trained at the Police College, Hendon, and the detective classes held in Ceylon, are responsible for a considerable proportion of this increase. It is gratifying to record that "clues" were given to the police in a number of cases in which the Department was consulted.

POISONING CASES.—The number of cases investigated has remained remarkably constant from year to year, varying only from 88 to 95 in the past five years. In 42 of the 90 cases submitted in 1938 poison was detected. The poisons included arsenic, 10; potassium cyanide or prussic acid, 9; mercury, 4; strychnine, 4; acetic acid, sulphuric acid and croton oil, 3 each; copper salts, alcohol, quinine salts and powdered glass, 2 each. The remainder included a mydriatic alkaloid, nitric acid, potassium permanganate, salicylic acid, yellow phosphorus, *Petchia zeylanica* and an unidentified aromatic oil. In a case of abortion a hollow stalk, which contained a paste of arsenic and copper sulphate and smelt strongly of asafoetida, was used. The cases in which quinine was found were also concerned with abortion.

Identification of Glass.—In one of the poisoning cases in which powdered glass had been used it was possible to show that the particles separated from a curry had the same specific gravity as glass from the suspected source.

The method of determining the specific gravity of fragments of glass by immersion in a liquid the gravity of which can be varied at will has proved very useful. In a case in which a car had driven off after a fatal accident fragments of glass found at the scene corresponded with the glass of the broken headlights.

In another case it was possible to fit together a number of small fragments of glass found at the scene of a gang robbery, and to show that they formed part of the broken glass of a torch in the possession of one of the accused.

SHOOTING CASES.—The number of exhibits (173) examined in connection with 54 cases showed a very large increase over former years. In many cases it was possible to demonstrate that a cartridge had been fired from a gun produced. It was also possible to exclude a number of guns. By arrangement with the Customs the Department is informed whenever a new brand of cartridge is imported, and the reference collection thus formed has proved its value during the year.

EXPLOSIVES AND FIREWORKS.—Fifty-six samples of crackers and 114 of explosive mixtures and fireworks were analysed, and some of these were as dangerous as hand-grenades. Aluminium powder has a large and legitimate use in pyrotechnics for the production of golden rain, but in the locally manufactured fireworks its sole use is to produce a high explosive (*cf.* ANALYST, 1938, **63**, 502). Arsenic sulphide in conjunction with chlorate and sulphur is another popular, though prohibited, mixture. The state of the car of the Magistrate of Colombo, after a bomb filled with this explosive had been thrown at him, provides ample justification of this prohibition. The magistrate probably owes his life to the indifferent aim of his assailant.

The Mechanical Testing of Bituminous Road Materials*

THE Road (Materials and Construction) Research Board is appointed by the Department of Scientific and Industrial Research to advise generally on the conduct of research on road materials. The Ministry of Transport is similarly responsible for full-scale road experiments. A necessary preliminary to the study of the physical and mechanical properties of bituminous surfacings was obviously an examination of the literature, and a critical digest of this is given in the present Report.

The Introduction deals with the classification of bituminous road materials, their characteristic properties, the purpose of mechanical tests, and the classification of mechanical tests.

Part II discusses the principal mechanical tests under the following heads: (i) Tests involving plastic properties of the materials. (ii) Tests involving elastic properties of the materials. (iii) Miscellaneous tests. (iv) Service tests.

Part III, on Mechanical Tests and Road Practice, includes (i) Employment of mechanical tests in mixture design. (ii) Mechanical tests and the macadam types of surfacing.

Part IV contains a critical study of mechanical tests and discusses the influence of test conditions on the results obtained. It includes: (i) Effect of degree of compaction of the specimen. (ii) Effect of size of specimen. (iii) Effect of rate of loading. (iv) Effect of ageing of specimens. (v) Effect of temperature of test. (vi) Degree of reproducibility of results.

The following classification has been adopted in order to give some idea of current practice in bituminous road construction. Four main divisions may be distinguished according to the grading of the mineral matter, *viz.*

I. Tar or bituminous macadams, containing large aggregate only.
II. Mixtures containing a preponderance of large aggregate.
III. Mixtures containing a preponderance of fine material.
IV. Mixtures containing fine material only.

In practice the various groups suggested by these divisions tend to merge into each other, but the divisions are of assistance in indicating the broad outlines of current practice. The Report gives brief descriptions of the various types of construction falling into each main division.

The various papers discussed are tabulated in an alphabetical list of references, which contains 137 descriptive entries.

* Road Research and Experiment. Special Report No. 1. A Survey of the Literature. By T. Lonsdale, M.Sc., Ph.D., F.Inst.P. Department of Scientific and Industrial Research and Ministry of Transport. Pp. 47. H.M. Stationery Office, York House, Kingsway, London, W.C.2. 1939. Price 1s. net.

Nomenclature of Hormones*

AT the Third International Conference on the Standardisation of Hormones, held at Geneva, August 11th to 13th, 1938, the question of nomenclature was considered, and it was agreed:

(1) That the fully descriptive term "anterior lobe of the pituitary gland" should be adopted for reference to the organ in question in the records of the Conference.

No general agreement was reached in the discussion of the respective claims of the suffixes "trophic" and "tropic" in the construction of descriptive terms for reference to the active principles with which the Conference had dealt.

It was agreed:

(2) That the use of the forms with the suffix "trophic" or "tropic" in the official English and French versions of the discussions and decisions of the Conference would be acceptable to members of the Conference. It was further agreed that the term "tropic" might, if necessary, be used with appropriate modifications in translations into other languages.

It was agreed:

(3) That the Conference recommend the adoption, for general scientific use, of the term "gonadotrop(h)in" for reference to principles with gonadotrophic action, with the addition, in each case, of a suitable adjective or descriptive phrase, to indicate the source of origin of the particular principle to which reference is made.

(4) That the Conference recommend the adoption, for general use, of the terms "corticotrop(h)in" and "thyrotrop(h)in," to denote the active principles of the anterior lobe of the pituitary gland acting on the adrenal cortex and on the thyroid gland, respectively.

British Standards Institution

THE following Standard Specifications have been issued†:

NO. 838—1939. BRITISH STANDARD METHOD OF TEST FOR THE TOXICITY OF WOOD PRESERVATIVES TO FUNGI.

The most important of the factors upon which the efficacy of a wood preservative depends are:—(1) toxicity to wood-destroying fungi; (2) penetrating power; (3) permanence. The present Specification deals only with toxicity, and stress is laid upon the fact that it is not possible to estimate from toxicity tests alone the value of a wood preservative when exposed out-of-doors to the influence of the weather.

Attempts have recently been made to standardise two types of toxicity tests: (1) the so-called agar or Petri dish method (Schmitz *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 361), and the wood-block method, for which an international standardised method has been prescribed (Liese *et al.*, *Z. angew. Chem.*, 1935, [21], 48). The relative value of the two methods has been studied by Findlay (*Ann. Appl. Biol.*, 1932, **19**, [2], 271), who concludes that the agar test gives only preliminary results. It is of value for comparing the toxicities of substances of similar character, *e.g.* phenols, but gives erroneous results with substances differing widely in chemical composition. At the Berlin Conference the wood-block method was considered the most suitable means for estimating the toxicity of a wood preservative to fungi, and this method is recommended for adoption as the British Standard Method.

The Specification gives an outline of the principles of the test, and a description, with dimensions, of the standard (Kolle) culture flask proposed by the International Committee. The flask used at the Forest Product Research Laboratory is somewhat larger, and has a reservoir in the neck for water. The standard medium consists of 50 g. of malt extract (B.P. quality) and 20 g. of agar per litre. About 40 ml. of this medium are sterilised in the culture flask for 20 minutes at 15 lb. gauge pressure, or by steaming at atmospheric pressure for 30 minutes on each of three successive days. The medium is inoculated with the test fungus within a few days after preparation, and the test blocks are infected by placing them over the cultures in the

* *Bulletin of the Health Organisation of the League of Nations*, Vol. VII, Extract No. 32, pp. 898–899.

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

flasks in such a way that the wood comes in contact with the aerial mycelium but not with the medium itself.

The test fungi employed are: *Coniophora cerebella* Pers. (Idaweiche strain); *Lentinus lepideus*, Fr. (= *L. Squamosus*), which is particularly resistant to tar preservatives; *Poria vaporaria* (Pers.), Fr. (Eberswalde strain); for tests on beech, *Polystictus versicolor* (Linn.), Fr., should be employed.

The species of wood ordinarily used are: (1) the sapwood of Scots pine (*Pinus sylvestris*, and (2) the outer wood (*i.e.* within 3 in. of the inside of the bark) of beech (*Fagus sylvatica*). The blocks (of standard dimensions) are dried, sterilised for about 18 hours in an oven at 100°–105° C., and weighed as aseptically as possible to the nearest centigram ("initial weight"), after which they are impregnated for about 2 hours with the preservative under reduced pressure, then allowed to drain for not longer than one minute, and again weighed. The concentration of preservative is calculated as kg. per cb. metre of wood. The test blocks are exposed to fungal attack for three months, and the amount of decay is calculated from the loss in dry weight of the wood, determined under specified conditions.

The Specification gives examples of toxic limits (kg. per $m.^3$) obtained with samples of creosote and commercial sodium fluoride, and an example of evaluation of test results.

No. 839—1939. British Standard Specification for Veterinary Cod-liver Oil.

This Specification forms part of a series of British Standards for Marine Animal and Fish Oils, the preparation of which was authorised by the Chemical Divisional Council.

For the purpose of the Specification, Veterinary Cod-liver Oil is defined as the product obtained from the fresh, iced or otherwise suitably preserved livers of the cod, *Gadus morrhua* Linn, and other species of *Gadus*.

The oil, when kept for 24 hours at a temperature of 16° C., shall be a clear yellow liquid and shall be free from any foreign matter. The odour shall be slightly fishy but not rancid, and the taste shall be bland and slightly fishy.

Colour.—The colour of the oil, filtered at 20° C., when measured through a 1-in. cell, shall be not deeper than a colour equivalent to a combination of 12 yellow and 2·7 red units on the Lovibond colour scale. (*Note.*—The number of glasses shall be the same on each side of the Lovibond instrument, colourless glasses being placed in front of the oil to secure this if necessary.)

Specific gravity and density.—The sp.gr. of the oil at 15·5°/15·5° C. shall not be lower than 0·922 nor higher than 0·929. Densities at 20° C. (*i.e.* weight in air of unit volume of oil) may be obtained by subtracting 0·0018 from the corresponding sp.gr. at 20° C.

Refractive index.—The value for the D line at 20° C. shall be not lower than 1·478 nor higher than 1·482.

Iodine value.—This shall be not lower than 150 nor higher than 178 when determined by the Wijs method.

Saponification value.—When determined in the specified manner this shall not be lower than 180 nor higher than 190.

Acidity.—The acidity (expressed as per cent. of oleic acid) shall not exceed 1 per cent. unless otherwise declared, and in any event shall not exceed 3 per cent. when determined by the specified method.

Unsaponifiable matter.—The oil shall not contain more than 1·5 per cent., unless otherwise agreed, and in no case more than 1·7 per cent., as determined by the Society of Public Analysts' method.

Vitamin potency.—The vitamin potency of the oil shall be not less than 500 units of vitamin A per gramme, and 50 units of vitamin D per gramme. All declarations of vitamins A and D potencies shall be made in International units per gramme, and the oil shall be tested for vitamin potencies in accordance with the specified methods (B.P. 1932, pp. 86–91; pp. 597–600, incorporating amendments in Addendum 1936 to B.P. 1932).

Blue value.—If the oil is submitted to the antimony trichloride test described (based on the B.P. method, 1932, pp. 596–7, and deleted by the Addendum, 1936), the blue value shall not be lower than 6. If the blue value of the oil is declared, it shall have been carried out by the method described.

The blue value of Veterinary Cod-liver Oil is not to be taken as a measure of the vitamin A content, but only as an approximation thereto. If a discrepancy arises between the vitamin A values suggested by this method and by the biological method, the value determined by the biological method shall be accepted.

Sampling and size of sample.—Representative samples, each measuring not less than 400 ml., shall be taken, wherever possible in triplicate, from original containers or from the bulk, and shall be packed in clean, dry, air-tight, non-absorbent containers, on which the sample has no action. The containers shall be of such size that they are nearly filled by the sample. Each sample container so filled shall be marked with full details and date of sampling.

Recommended methods for sampling fats and fatty oils, are given in B.S. 627.—1935.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of the Volatile Products Liberated from Stored Fruit. F. Gerhardt and B. D. Ezell. (*J. Agric. Res.*, 1939, **58**, 493–503.)—Acetaldehyde, identified as one of the more common and abundant volatile products of fruit metabolism, has been taken as representative of the odorous constituents formed in a specific type of physiological metabolism under the conditions of handling practice or storage temperature. The method used for the determination of the total oxidisable volatile compounds emanating from stored fruit was based on their absorption in conc. sulphuric acid, oxidation with ceric sulphate and evaluation in terms of ceric sulphate reduced. Small absorption towers filled with glass beads and fitted with 250-ml. extraction flasks were used as gas scrubbers, the flasks were charged with 25 ml. of sulphuric acid, and the volatile substances were conveyed in an air-stream of 10 litres per hour. After the absorption the beads were rinsed with 75 ml. of water into the extraction flasks, $N/10$ ceric sulphate solution was added (50–100 ml.), and the flasks were heated over a boiling water-bath for 2 hours, after which their contents were transferred to 500-ml. flasks and made up to the mark. Aliquot portions (100 ml.) were diluted with 250 ml. of water, 0·3 to 0·5 g. of potassium iodide was added, and the solutions were titrated with $N/10$ sodium thiosulphate solution. The average recovery of acetaldehyde was 97·2 per cent. Applications of the method included measurements of the volatile substances in air from various stores, inter-relation of respiration and liberation of volatile substances from Golden Delicious apples, and the influence of certain fruit-rot fungi on the emanation of volatile substances. D. G. H.

Bromide-Content of Fruits and Vegetables following Fumigation with Methyl Bromide. H. C. Dudley. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 259–261.)—The samples were fumigated for 2 to 24 hours at atmospheric pressure and at 20° to 25° C. with 2 lb. of methyl bromide in 1000 cb.ft. of air. Tests for bromide were made immediately afterwards and also at intervals after exposure to fresh air. The samples absorbed up to 6 or 7 mg. of methyl bromide per 100 g., but generally much of this was lost in 48 hours after fumigation. The following are examples from the results.

	Mg. Bromine per 100 g.		
	before fumigation	immediately after	48 hours after
Green beans	0·54	7·22	4·08
Dried peaches	1·44	2·31	1·60
Apples (fresh)	none	0·30	0·27

The method of determination involved digestion of the sample with alcoholic potash, followed by evaporation and ashing of the residue; bromine was liberated by a chromic-sulphuric acid mixture, carried over by air aspiration into potassium iodide solution, and determined by titration with thiosulphate, on the lines of the method of Baughman and Skinner (*Ind. Eng. Chem.*, 1919, **11**, 954; Abst., ANALYST, **1919, 44, 417**). S. G. C.

Methods of Analysis of Molasses used in the Fermentation Industries. W. A. Davis. (Research Labs., Distillers Company, Ltd., Epsom, pp. 16.)—This description of methods adopted in the Research Department of the Distillers Company is published as "a contribution to the unification of methods of sugar analysis." For distilleries the value of a molasses depends mainly on the yield of alcohol obtainable from it in practice. A procedure for determining this by a fermentation test, occupying a number of days, is described. Methods are described also for determining, by ordinary analysis, the content of total sugars, but this is not a satisfactory measure of the yield of alcohol, as it includes a variable proportion of unfermentable sugars ("glutose"), the amount of which, calculated as invert sugar, has been found to range from 3 to 6 per cent. in blackstrap molasses, from 2 to 3 per cent. in "high-test" molasses (containing 70 to 80 per cent. of total sugars) and from 0·4 to 1 per cent. in beet molasses. These percentages probably represent about twice as much in actual material, for the reducing power of the unfermentable sugars is probably only about half that of invert sugar, but it is usual to express them in terms of invert sugar. The proportion of unfermentable sugars is variable even amongst molasses of the same type; the judging of distilling value from the content of total sugars therefore demands caution and experience. A useful value described is the *fermentation value*, defined as the actual yield of alcohol, expressed as a percentage of the yield theoretically obtainable if the total sugars present (after inversion) could be transformed in accordance with the equation: $C_6H_{12}O_6 \rightarrow 2C_2H_6O + 2CO_2$; this value may range between 80 and 90 per cent.

Determination of Total Sugars.—Reducing methods only are used, polarimetric methods being considered less accurate with molasses. Reducers are determined as invert sugar with Fehling solution, before and after inversion. Inversion is carried out on 1 per cent. solutions, under Clerget–Herzfeld conditions but with 10 minutes' total heating in a bath at 70° C. The molasses solutions are not clarified before analysis, on the ground that without clarification the results are sufficiently close to those obtained after clarification with neutral lead acetate followed by de-leading and decalcification with potassium oxalate (*cf.* Eynon and Lane, *J. Soc. Chem. Ind.*, 1923, 466T). For the determination of total sugar as invert sugar after inversion, the Lane–Eynon method is used, with 10 ml. of Fehling solution and a sugar concentration of 0·2 to 0·3 per cent. For the determination of invert sugar before inversion, when the proportion in the molasses exceeds 20 per cent., the same method may be used or alternatively the Brown, Morris and Millar gravimetric method (Allen's *Commercial Organic Analysis*, 5th Ed., Vol. I, 397). With molasses containing less than 20 per cent. of invert sugar (and therefore with all beet molasses) this original invert sugar is always determined by the Brown, Morris and Millar method. This determination is carried out on 50 ml. of 1 per cent. molasses solution, and the precipitated cuprous oxide, collected in a Gooch crucible, is oxidised by moderate ignition and weighed as cupric oxide. If the weight (corrected by a blank) is less than 0·245 g. it is multiplied by 81·5 to obtain the percentage of invert sugar in the molasses; the calculation for larger weights of cupric oxide is in accordance with the Table of Brown, Morris and Millar.

Determination of Spirit Yield and Unfermentable Sugars.—A solution of 200 g. of blackstrap (or 150 g. of high-test) molasses in about 400 to 500 ml. of tap-water is adjusted to *p*H 4·4–4·6 (high-test to *p*H 4·9 to 5·1) with sulphuric acid (usually 15 to 20 ml. of 2 *N* acid for 200 g. of cane molasses, and 0 to 4 ml. for 150 g. of high-test molasses) and treated with 25 ml. of 4 per cent. diammonium phosphate solution adjusted to *p*H 5 with sulphuric acid. The liquid is diluted to 1 litre and weighed. Two 400-ml. lots are weighed into two 750-ml. conical flasks. Four drops extra are then added to each, and from each flask 2 ml. are pipetted into a test-tube and 20 ml. into a 100-ml. conical flask. These (four) small receptacles are then plugged with cotton wool and they, and the 750-ml. flasks fitted with empty liquid traps (to contain conc. sulphuric acid later), are sterilised by steaming. When cooled, the test-tubes are seeded with a pure culture (agar slope) of the yeast actually employed in the distillery, and after incubation at 33° C. for 24 hours their contents are transferred to the 100-ml. flasks, which are similarly incubated and their contents after 24 hours transferred to the 750-ml. flasks. These, closed by their traps, into which 2·5 ml. of conc. sulphuric acid are introduced, are weighed to 0·01 g. and incubated at 33° C. Each is re-weighed after 3 days and then day by day until the loss in 24 hours does not exceed 0·05 g. After cooling, the acid from the trap is poured into a small flask containing ice, and nearly neutralised with 5·0 ml. of 50 per cent. sodium hydroxide solution, and it is then added to the fermented liquid, which is made up to 500 ml. On an aliquot part (100 ml.), alcohol is determined pycnometrically after being carefully distilled twice, first from the somewhat acid liquor, and again after being made slightly alkaline with sodium hydroxide. Duplicate results agree to within about 1 in 500 of the alcohol found.

To determine unfermentable sugar, a further aliquot part of the final fermented solution is neutralised and, after addition of alumina cream, filtered, and the apparent invert sugar is determined by the Brown, Morris and Millar method. Pentoses and pentosans may be determined by Kröber's method (*id.* p. 498) on a portion of the same filtered solution after removal of alcohol by evaporation.

Identification and Determination of Lactic Acid in Alcoholic Fermented Liquids. M. Niculescu. (*Z. anal. Chem.*, 1939, **116**, 175–183.)—The application of three methods to the detection of lactic acid in fermented liquids was studied. The reaction of Denigès (*Z. anal. Chem.*, 1911, **50**, 189), in which the lactic acid is converted by treatment with conc. sulphuric acid into aldehyde, which is identified by its reaction with guaiacol, was found to be unsuitable in the presence of small amounts of dextrose which invariably accompanied the lactic acid separated by extraction with ether. Detection by conversion into zinc lactate also proved unsuitable, since the crystals were never typical, owing to the presence of other substances. The reaction of Uffelmann (*Z. anal. Chem.*, 1888, **27**, 543), although not specific, was found most suitable for the detection of lactic acid separated from fermented liquids by extraction with ether. The reagent is prepared immediately before use by mixing 10 ml. of 4 per cent. phenol solution with 20 ml. of water and one drop of ferric chloride solution. Its amethyst-blue colour becomes yellow in presence of lactic acid. Gravimetric methods depending upon

conversion into the zinc or calcium salt, volumetric methods depending upon titration of the separated acid, and optical methods were all found to involve appreciable errors caused by impurities in the separated lactic acid. The method finally recommended is based upon that of Fürth and Charnass (*Biochem. Z.*, 1910, **26**, 199), as modified by Tanaka and Endo (*ibid.*, 1929, **210**, 120), in which the lactic acid is oxidised to aldehyde, which is determined by an iodimetric method. The solution, containing about 5 mg. of lactic acid, is mixed with 35 ml. of 10 per cent. sulphuric acid and diluted to 150 ml. with water. After addition of a little talc, the mixture is heated in a flask provided with a dropping funnel and connected through a vertical water-condenser with a long-stemmed adapter leading into a filter-flask, the stopper of which also carries a tube through which sodium bisulphite solution can be introduced from a burette. The side-tube of the filter-flask is joined by rubber tubing carrying a screw-clip to a long tube bent twice at right angles and dipping into water, which acts as a seal. While the liquid in the distillation flask is being heated, sodium bicarbonate solution is run in slowly from the dropping-funnel until by the time boiling has begun the air in the apparatus has been replaced by carbon dioxide. A measured amount of sodium bisulphite solution (2·5 g. dissolved in a litre of water saturated with carbon dioxide) is delivered into the filter-flask from the burette until the outlet of the adapter is immersed. When the liquid begins to boil potassium permanganate solution (approx. 0·005 *M*) is run, drop by drop, from the dropping-funnel into the boiling solution until a pink colour persists. Distillation is continued for 30 minutes, any tendency of the bisulphite solution to rise in the adapter being checked by the cautious addition of sodium bicarbonate solution through the dropping-funnel. When distillation is complete the filter-flask is disconnected from the adapter (which is rinsed with a little water) and closed by means of a stopper and the screw-clip, the tube leading to the water-seal being removed. The liquid is agitated frequently during 30 minutes to promote reaction between the aldehyde and the bisulphite solution, and the uncombined sodium bisulphite is finally titrated with *N*/100 iodine solution, starch being used as indicator. The titre of the bisulphite solution is then determined, and the difference corresponding with the sodium bisulphite equivalent to the aldehyde formed is calculated into lactic acid. The method differs from that of Tanaka and Endo (*loc. cit.*) in that the sodium bisulphite solution is not introduced into the apparatus until all the air has been expelled. It was found that if the air is expelled through the bisulphite solution, oxidation occurred and erroneous results were obtained. Before its determination the lactic acid must be separated from the fermented liquid. Volatile substances are removed by steam-distillation and the residue, suitably concentrated, is kneaded into a firm mass with calcium sulphate and extracted with ether in a Soxhlet apparatus. Alternatively, the fermented liquid may be treated with finely powdered ammonium sulphate, allowed to stand for 24 hours, filtered, acidified with dilute sulphuric acid and extracted five times with the same portion of amyl alcohol, the lactic acid being recovered from the amyl alcohol after each extraction by means of 2 per cent. sodium carbonate solution. The combined sodium carbonate extracts are mixed with a little dimethylamidoazobenzene indicator, slightly acidified with dilute sulphuric acid and exactly neutralised with sodium

carbonate solution. Suspended amyl alcohol and the indicator are then removed by steam-distillation. This method is rapid, but extraction with ether in a Soxhlet apparatus gives somewhat higher and more accurate results. A. O. J.

Oxidation in Beers. I. Simplified Method of Measurement. P. P. Gray and I. Stone. (*J. Inst. Brewing*, 1939, **45**, 253–263.)—The probability of a correlation between the stability of a beer and its rH value is discussed (*cf.* De Clerck, *id.*, 1934, **40**, 292, 407). It is concluded that such a relationship might be misleading, inasmuch as the rH value is derived theoretically from ideal, thermodynamically-reversible systems of known characteristics and in a state of delicate equilibrium, and with beer these conditions are not fully satisfied. Moreover, the usual electrometric technique is unsuitable for ordinary routine control in the brewery, and a simple colorimetric method which, it is considered, represents an empirical approximation of the oxidation-reduction capacity is preferred. The principle used is the measurement of the rate of partial (80 per cent.) decolorisation of a suitable indicator (*cf.* Hartong, *id.*, 1935, **41**, 116; *Wallerstein Lab. Comm.*, 1937, No. 1, 5). Sufficient gas is removed from the beer to eliminate excessive frothing, and three 10-ml. portions at 25° C. are placed in similar test-tubes, distilled water being introduced into two similar tubes. In a sixth tube are put 0·05 ml. of a 0·005 *M* solution of the indicator (see below), and 10 ml. of a phosphate buffer solution of pH 4·4; 0·25 ml. of the indicator solution is then put into one of the tubes containing the sample, the contents are mixed rapidly, and a stop-watch is at once started. The tubes are placed in a "block" type comparator, so that the three holes in the back row contain tubes of (1) water, (2) water, and (3) the indicator plus buffer, respectively, whilst in the corresponding holes in the front the tubes contain (1) beer, (2) beer plus indicator, and (3) beer, respectively. The time in seconds (known as the "indicator time test" or *I.T.T.*) is noted when the colour of the light transmitted by the centre pair of tubes (containing beer plus indicator and water) matches that transmitted by the pair containing indicator plus buffer and beer. The other pair of tubes serve as a visual aid to colour matching. The indicator is prepared by weighing out a little more than 150 mg. of the powdered sodium salt of 2.6–dichloro-benzenone-indophenol, and stirring this thoroughly with hot water; when cold, the solution is diluted to 100 ml. and filtered through a No. 40 Whatman paper. It is then standardised by titrating a mixture of 10 ml. of solution, 4 ml. of a freshly-prepared 25 per cent. solution of potassium iodide, and 2 ml. of dilute sulphuric acid (1 + 6) with 0·01 *N* sodium thiosulphate solution, and starch as indicator. The concentration, in mg., of indicator per 100 ml. of solution is obtained by multiplying the titration volume by 14·5, and this figure is used to adjust the strength of the solution of indicator to 145 mg. per 100 ml., *i.e.* approximately 0·005 *M*. The indicator should be kept in small, completely-filled and stoppered glass bottles, which are stored in a refrigerator, and it should be prepared every week, and standardised when required for use. For convenience in control work permanent colour standards may be used, as these keep for at least a year if sealed in Pyrex test-tubes and stored in the dark. They are prepared from (*a*) a solution of 5·0 g. of roseo-cobaltic chloride, $[CoCl_3(NH_3)_5,H_2O]$, and 100·0 ml. of conc. ammonium hydroxide in sufficient water to make 1 litre; (*b*) a

solution of 5·0 g. of crystalline copper sulphate and 100·0 ml. of conc. ammonium hydroxide in sufficient water to make 1 litre. Colours corresponding with 80 and 50 per cent. decolorisation of the indicator are prepared by mixing 230 ml. of (*a*) with 70 ml. each of (*b*) and conc. ammonia; and from 540 ml. of (*a*) and 150 ml. of (*b*) with 31 ml. of conc. ammonia (see below), respectively. The former corresponds with the desired end-point. The characteristics and method of standardisation of the indicator selected have been investigated in connection with the determination of vitamin C. Since the oxidation-reduction potential range within which it is decolorised is well above that of beer, those constituents of beer which are susceptible to oxidation by air act as reducing agents towards it; the rate at which this occurs is the value required, and corresponds with the state of oxidation of the beer. The results are not affected immediately by dissolved oxygen, owing to the high oxidation-reduction potential range. The *I.T.T.* values for different beers range from 50 to over 3000 seconds, and a close connection has been traced between them and the colloidal stability and changes in flavour of the beers. Thus, beers which have been bottled with air develop oxidation hazes and have *I.T.T.* values which increase in time as such oxidation proceeds (*cf.* Wallerstein, *loc. cit.*). A logarithmic relationship was found to exist between the *I.T.T.* value and results obtained by the usual method for the colorimetric determination of the *r*H value (*cf.* Laufer, *J. Inst. Brewing*, 1936, **42**, 141). The slope of the curve is such that an *r*H range of 12 to 16 corresponds with an *I.T.T.* range of 50 to 3000 seconds, and this indicates the sensitive nature of the present test; moreover, unlike the other methods it does not necessitate a waiting period of 24 hours before making the test, so that the results can indicate the condition of the beer at any desired time. The indicator used varies in colour with the *p*H, being red at *p*H 4·6 and blue at *p*H 6·0; the use of a buffer solution (see above) is therefore necessary, and the value of 4·4 is chosen because it corresponds with that of beer itself. Higher values should be used for worts, although data are given which show that within the normal range of *p*H values encountered in brewing operation (3·2 to 5·8) as produced by the addition of acid or alkali to beer, the variation in the *I.T.T.* value falls within the limits of experimental error. Data also show that there is a linear relationship between the percentage decolorisation of the indicator and the logarithm of the time for which it is allowed to react. Hence with highly-oxidised samples (which have very low *I.T.T.* values) it is more convenient to measure the time required to decolorise the indicator to the extent of 50 per cent. and to find the 80 per cent. value (*i.e.* the *I.T.T.* value) by extrapolation; the average extrapolation factor, as obtained from the graphs, is then 7. For such samples the comparison standard is prepared by adding 0·125 ml. of indicator to 10 ml. of buffer solution, and the corresponding permanent colour standard is given above. J. G.

Direct Determination of Chloride in Wine and its Occurrence in Bavarian and other Wines. H. Grohmann. (*Z. Unters. Lebensm.*, 1939, **77**, 482–488.)—The wine (100 ml.) is treated with 50 ml. of cold saturated barium hydroxide solution, diluted with water to 200 ml. and filtered, and 100 ml. of the filtrate are treated with 20 ml. of dilute nitric acid (1 : 5) and 2 or 3 ml. of saturated

potassium permanganate solution. After a few minutes, when the violet colour will have disappeared, the mixture is treated with 2 ml. of 3 per cent. hydrogen peroxide solution, which causes complete decolorisation. After the addition of 10 ml. of 10 per cent. ferric nitrate solution (ferric alum cannot be used in the presence of barium) and 20 ml. of ether (which promotes curdling of the silver chloride), $N/10$ silver nitrate solution is added and the excess is titrated with $N/10$ potassium thiocyanate solution in the usual manner until a red colour persisting for at least 5 seconds is formed. The accuracy of the method was established by the addition of known amounts of sodium chloride to wines of known chlorine-content. Authentic samples of wine from the Bavarian Palatinate were examined by this method. For red wines (18 samples) the chlorine-content varied from 22·7 to 85·1 mg. per litre, and for white wines (91 samples) from 14·2 to 138·3 mg. per litre, and in only 5 of these 109 samples did the chlorine-content exceed 90 mg. per litre. The range for Bavarian wines may therefore be taken as 10 to 90 mg. per litre and the values found by Halenke and Möslinger (König, *Chemie der menschlichen Nahrungs- und Genussmittel*, **2**, 1288) are confirmed. No explanation can be suggested for the high values shown by a few individual samples. The presence of more than 150 mg. of chlorine per litre may be regarded as a definite indication of adulteration either by the addition of substances containing chloride or by admixture with other wine. The chlorine-content of a number of foreign wines varied from 106·4 to 595·6 mg. per litre. The reason for these high values is not known. It has been suggested that they may be due to the use of grapes grown in an excessively saline soil, to deposits on the grapes of salt from wind laden with sea-spray, to the use of pest-control materials prepared from sea-water, or to absorption of salt from casks which have been filled with sea-water. In the literature the chlorine-content of wine is frequently expressed as sodium chloride, and Merz (*Wein und Rebe*, **10**, 103) suggests that the chlorine-content may be inferred from the sodium-content. Determinations of the sodium-content by the method of Reichard (*Z. Unters. Lebensm.*, 1936, **71**, 501) showed that sodium and chlorine are not present in equivalent amounts in wine. A. O. J.

Brandy. P. Valaer. (*Ind. Eng. Chem.*, 1939, **31**, 339–353; *J. Inst. Brewing*, 1939, **45**, 266–267.)—The manufacture, characteristics and composition of brandies prepared from various fruits in America and other countries are discussed. Three classes of brandy are manufactured in California:—(*a*) fortifying brandy, which is distilled to a proof-content of 180 to 190 per cent. (U.S.A.) and stored in metal drums; (*b*) commercial beverage brandy, which is distilled below 175 per cent. proof and stored in barrels at 102 per cent. proof; (*c*) "grappa" brandy prepared from pressed pomace which, after a long secondary fermentation, is mixed with grape residues, lees, etc., and distilled to yield less than 160 per cent. proof. American grape brandy is distilled in continuous stills, and then coloured with caramel and matured in new white-wood casks, whilst brandies from other fruits (and especially from apples) are usually prepared in pot stills and aged in charred-wood casks. All genuine brandies contain a little methyl alcohol, *viz.* less than 0·05 and about 0·10 per cent. in grape and other fruit brandies, respectively. The esters, however, are the most important constituents. They are present in brandy in greater

proportions than in any other spirit, and determine the flavour and general characterisitics of the product. In the maturing process ethyl acetate is produced the most rapidly and most abundantly, but the higher esters, although they are formed more slowly, are more important from the point of view of flavour. American grape and other fruit brandies differ from French cognac in that they have the aroma of the original fruit with an unmistakable natural flavour which is enhanced by maturation, whilst the flavour of cognac bears no resemblance to that of the fruit from which it is derived. French brandies contain about 0·02 per cent. of a clear yellow oil having a powerful odour of cognac; this is a natural ingredient and is absent from all other types of brandy. The flavour of brandy is the most important characteristic which determines its quality, but it is affected adversely by the use of impure water, and by contact with certain metals and unsuitable wooden containers. J. G.

Nicotine from Tobacco. C. Pyriki. (*Z. Unters. Lebensm.*, 1939, **77**, 379–385.)—It has been observed (Waser and Stahli, *Z. Unters. Lebensm.*, 1932, **64**, 740; Pyriki, *Pharm. Zentralh.*, 1933, **74**, 253; Hammerle and Weber, *Mitt. Lebensmittelunters.*, 1936, **27**, 46) that when tobacco is steam-distilled with magnesium oxide until no further nicotine is evolved, an additional evolution occurs if distillation is continued after the addition of sodium hydroxide or potassium carbonate. This evolution, which occurs somewhat slowly, can be hastened by the addition of hydrogen peroxide to the alkaline liquid. The present work was undertaken to determine what portion of the total nicotine obtainable is originally present in the tobacco and what portion is produced by decomposition of other substances. Experiments were made with finely powdered Kentucky and Java tobacco. An aqueous extract of each variety was steam-distilled after the addition of sodium chloride and sodium hydroxide until the distillate was free from nicotine, as indicated by the reaction with silicotungstic acid. Distillation was continued, and the distillate now obtained was acidified, concentrated, treated with picric acid, and left for four weeks in an ice-chest to promote the deposition of nicotine dipicrate. Only a very small amount of the dipicrate was obtained. After the lapse of one month the alkaline liquid was again distilled and the dipicrate was prepared; a considerable amount was now obtained. This procedure was repeated at monthly intervals until 19 fractions had been obtained from each variety of tobacco. The fractions were combined, and the dipicrate was purified by recovery of its nicotine, which was re-converted into the dipicrate. Nicotine was again liberated from the pure dipicrate by distillation with magnesium oxide, and by determination of its optical rotatory power in toluene solution it was proved to consist mainly of inactive racemic nicotine. This was confirmed by the determination of the m.p. of its picrolonate, which was 232·5 to 233° C., whereas the m.p. of *l*-nicotine picrolonate is 223° to 224° C. Control experiments with pure *l*-nicotine subjected to the same procedure showed that it suffered no change either in its optical activity or in the m.p. of its picrolonate. It is concluded therefore that the additional nicotine obtained gradually by the prolonged action of alkali is not present in the original tobacco, but is produced by the decomposition of other substances. An extract prepared from Kentucky tobacco was distilled

with sodium chloride and magnesium oxide. When the distillate gave only a slight turbidity with silicotungstic acid (fraction A) the receiver was changed and distillation was continued until the distillate ceased to answer to the test (fraction B). The residue was now made strongly alkaline with sodium hydroxide solution and distilled in steam until the reaction of the distillate with silicotungstic acid was again feeble (fraction C). Finally the distillation was continued with periodic addition of hydrogen peroxide until the distillate was again free from nicotine (fraction D). By determination of the m.p. of the dipicrates and picrolonates prepared from these fractions and of the optical activity of the fractions of nicotine recovered from them, it was found that A consisted of *l*-nicotine, that B was a mixture of other alkaloids volatile with difficulty, that C contained *l*-nicotine and nor-nicotine and that the nicotine in D was the racemic mixture resulting from decomposition of other constituents of tobacco. The final conclusion drawn was that the additional nicotine obtained by distillation with sodium hydroxide after the preliminary distillation with magnesium oxide was originally present in the tobacco and was not derived from other substances. Distillation with sodium hydroxide will therefore give the total nicotine-content of the tobacco. The nicotine obtained by prolonged action of sodium hydroxide (or more rapidly by distillation with sodium hydroxide and hydrogen peroxide) is mainly a racemic mixture formed by decomposition of other substances. If the tobacco contains other volatile alkaloids, these will appear both in the distillation with magnesium oxide and in that with sodium hydroxide. Their separation may be effected by the method of Koenig and his collaborators (Bömer, Juckenack and Tillmans, *Handbuch der Lebensmittelchemie*, 6th Ed., p. 296, Berlin, 1934).

A. O. J.

New Coupling Component for Sulphanilamide Determination. A. C. Bratton and E. K. Marshall. (*J. Biol. Chem.*, 1939, **128**, 537–550.)—Of 17 compounds examined as coupling agents for the estimation of sulphanilamide, N-(1-naphthyl)-ethylenediamine dihydrochloride was selected because it can be prepared in a highly pure state; it couples very rapidly, and the process is not influenced by *p*H in the range of 1 to 2. Further, the colour of the dye is not affected by variations of *p*H within this range, and the dye itself is very soluble. Two ml. of oxalated blood are measured into a flask, diluted with 30 ml. of 0·05 per cent. saponin solution, and after 1 or 2 minutes treated with 8 ml. of 15 per cent. trichloroacetic acid solution. The precipitate is filtered off, and 1 ml. of 0·1 per cent. sodium nitrite solution is added to 10 ml. of the filtrate, followed after 3 minutes by 1 ml. of 0·5 per cent. ammonium sulphamate solution, and after a further 2 minutes by 1 ml. of a 0·1 per cent. solution of N-(1-naphthyl)-ethylenediamine dihydrochloride. A comparison, within an hour, of the colour produced with that of an appropriate standard obtained by a similar procedure gives an estimate of the amount of free sulphanilamide in the blood. To estimate the total sulphanilamide, 10 ml. of the filtrate are treated with 0·5 ml. of 4 *N* hydrochloric acid, the mixture is heated in a boiling water-bath for 1 hour and then cooled, and the volume is adjusted to 10 ml. The subsequent procedure is as described above. To estimate sulphanilamide in urine, the protein-free sample is diluted so as to

contain 1 to 2 mg. per 100 ml., and 50 ml. of this diluted urine, plus 5 ml. of 4 *N* hydrochloric acid, are diluted to 100 ml. Ten ml. of this solution are treated as a blood filtrate for the determination of free sulphanilamide, and another portion of 10 ml. is treated for total sulphanilamide. If the urine contains protein, it is diluted and treated by the procedure for blood. The estimations can also be made with the aid of a photoelectric colorimeter, dilutions of blood (with water, saponin being unnecessary) of 1 : 50 or 1 : 100 being used. A filter, transmitting the maximum of light at 545$m\mu$ (the absorption maximum of the dye formed) must be used. Sulphanilamide can be estimated by this method in body fluids other than blood or urine. When tissues are to be analysed, the ground material is extracted in a Soxhlet apparatus with alcohol, and the extract is suitably diluted with water. Any sulphanilamide with a primary amino group or with a group convertible into one by hydrolysis, can be estimated, but high dilutions have to be used with sparingly soluble substances. The recovery of sulphanilamide itself added to blood was practically quantitative from a 1 : 50 dilution of the blood, but was only 90 per cent. complete from a 1 : 4 dilution. F. A. R.

Biochemical

Biochemical Importance of Unsaturated Fatty Acids. S. Skraup, F. Strieck and J. Schorn. (*Z. physiol. Chem.*, 1939, **259**, 1–18.)—The administration of natural fats to experimental animals is usually followed by a characteristic fall in the respiratory quotient, but when glycerides of fatty acids having an odd number of carbon atoms were fed to dogs, the respiratory quotient showed no such fall, nor was the amount of heat produced increased. The heat production did increase, however, when an unsaturated glyceride, for instance, triolein or the glyceride of heptadecenoic acid, was given together with the "odd" glycerides, showing that the fat was being metabolised more rapidly; the unsaturated glyceride alone did not increase the amount of heat produced. The "activator" function of unsaturated acids was confirmed by a different type of experiment in which groups of rats that had practically ceased growing were given saturated glycerides, with and without triolein. In nearly every instance the rats fed only with saturated glycerides lost weight, whilst those receiving also the unsaturated glycerides gained weight. The carcases of some of these rates were digested with sodium hydroxide solution and the fatty acids were isolated. It was found that more fat had been deposited in the rats receiving the saturated glycerides than in those receiving mixed glycerides, and that the fat from the former group of rats was more saturated than the fat from the latter. The experiments on dogs further showed that there was no significant difference between the accelerating effect of heptadecen-(8)-oic acid, oleic acid and nonadecen-(10)-oic acid, but that undecylenic acid and the acids of the elaidic acid series were inactive. The unsaturated acids are not catalysts in the accepted sense of that term; they are hydrogen acceptors, being themselves reduced—irreversibly—to saturated acids. It appears that the dictum "fat is burnt in the fire of carbohydrates" now requires the reservation that the unsaturated fatty acids serve as the "activators" or "initiators" of this fire. F. A. R.

Connection between Taste and Constitution of Carboxylic Acid Hydrazides and their Derivatives. J. J. Blanksma and H. A. Bakels. (*Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 497–513.)—The connection between taste and chemical constitution of malonyl and succinyl dihydrazides, the trihydrazide of citric acid, the hydrazides of ortho-, iso- and terephthalic acids and nuclear substituted 2-hydroxymethylbenzylhydrazides, was investigated. It was found that the sweet taste of malonyl dihydrazide disappeared on condensation with aldehydes and ketones, bitter substances being produced with acetone, methyl ethyl ketone and diethyl ketone, the taste being less pronounced the greater the size of the alkyl group. The acetyl derivative was also bitter. Similar results were obtained with the sweet tasting succinyl dihydrazide, whilst the very sweet trihydrazide of citric acid gave, on condensation with aromatic aldehydes and ketones, insoluble derivatives that were without taste. It was not found possible to prepare orthophthaldihydrazide; phthalhydrazide, which is tasteless, was obtained instead. Isophthal-dihydrazide and terephthaldihydrazide were found to be tasteless, but the two acetone derivatives were slightly bitter. The monomethylhydrazide and dimethylhydrazide of phthalic acid were tasteless. Phthalide, meconine and benzyl alcohol have bitter tastes; 6-nitro-phthalide was found to be less bitter than phthalide itself, whilst 6-amino-phthalide and 4-nitro- and 4-amino-meconine were tasteless. The 2-hydroxy-methyl-hydrazides obtained by treating meconine and phthalide with hydrazine also had bitter tastes, but benzhydrazide and its acetone and benzal derivatives were tasteless. Maximum bitterness was attained in this group with 5-nitro-2-hydroxy-methyl benzhydrazide. It thus appears that at least two .CO.NH.NH_2 groups must be present to produce a sweet taste, and that these groups must not be too far from one another in the molecule. A hydroxyl group apparently increases the sapophoric function of the hydrazine residue. Condensation of hydrazides with acetone in particular sometimes, but not always, results in bitter-tasting compounds. The introduction of a hydroxyl-methyl group also produces a bitter taste. F. A. R.

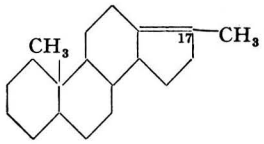
Micro-determination of Glutamic Acid. P. P. Cohen. (*Biochem. J.*, 1939, **33**, 551–558.)—Glutamic acid is converted by treatment with excess of chloramine-T into β-cyanopropionic acid, which is converted by acid hydrolysis into succinic acid; this can be estimated manometrically by means of a succin-oxidase preparation. By employing the right conditions in the first two stages of this series of reactions, a quantitative conversion of glutamic acid into succinic acid is obtained. Glutamic acid can be determined in tissue slices without the necessity of de-proteinising, but suspensions of minced tissue must first be freed from protein by the addition of two-thirds of their volume of 2/3 *N* sulphuric acid and one-third of their volume of 10 per cent. sodium tungstate solution. The solution to be analysed is adjusted to *p*H 4·7 by the addition of 1 to 1·5 ml. of citrate buffer solution (17·65 g. of sodium citrate, $Na_3C_6H_5O_7.2H_2O$, and 8·40 g. of citric acid, $C_6H_8O_7.H_2O$, in 50 ml.), 2 ml. of 10 per cent. chloramine-T solution are added, and the solutions are well mixed. They are then shaken at 40° C. for ten minutes, after which the flasks are placed in an ice-bath for 15 to 20 minutes to cause the precipitation of the *p*-toluene-sulphonamide formed

and the excess of chloramine-T. The solutions are filtered, the precipitate is washed with several small volumes of water, and the combined filtrate and washings are collected in large test-tubes. The solution is acidified with 4 ml. of 10 per cent. sulphuric acid and the β-cyanopropionic acid is extracted from the solution in a continuous extractor with ether. The contents of the flask are treated with 2·5 ml. of 0·1 *M* phosphate buffer solution (*p*H 7·4), and the ether is slowly distilled off. The remaining aqueous solution is then chilled to precipitate the *p*-toluenesulphonamide, which is filtered off. Sufficient conc. hydrochloric acid is added to the filtrate to bring the concentration of acid up to at least 12·5 per cent., and the tubes are then heated in a boiling water-bath for 15 minutes and cooled, and sodium hydroxide solution (50 to 60 per cent.) is added dropwise until the solution becomes hot. Then 0·5 ml. of 5 per cent. ammonium chloride solution is added to destroy traces of chloramine-T, and the solution is made alkaline to phenol red, a few drops of which are added to the solution. The remaining traces of *p*-toluenesulphonamide are removed by extraction in a continuous extractor with peroxide-free ether, after which the solution is made strongly acid to phenol red with 3 ml. of 10 per cent. sulphuric acid, and again extracted with peroxide-free ether. When the extraction of succinic acid is complete, 2 to 3 ml. of 0·1 *M* phosphate buffer solution are added to the ethereal solution followed by dropwise addition of 2 *N* sodium hydroxide solution until the reaction is neutral or slightly alkaline. The ether is distilled off, and the aqueous solution is concentrated to about 1 ml. and transferred with the aid of 0·5 to 2 ml. of 0·1 *M* phosphate buffer solution to a graduated flask. The succinic acid is then estimated according to the method of Krebs (*Biochem. J.*, 1937, **31**, 2095). The recovery of known amounts of glutamic acid from pure solutions varied from 91·5 to 98·2 per cent., and the recovery from protein solutions was of the same order. As little as 1 mg. of glutamic acid can be determined by the method, which is highly specific. No compound other than glutamine occurring in biological material has been found to react. F. A. R.

New Colour Reactions for the Steroids. H. Kägi and K. Miescher. (*Helv. Chim. Acta*, 1939, **22**, 683–697.)—On treating the two epimeric testosterones with bromine (or other halogen) in presence of a mineral acid, the *cis*, but not the *trans* compound, was found to give a red solution, and on examining a series of similar pairs of compounds the reaction was found to be a general one for all steroids possessing a 17-*cis* hydroxyl group, but no colour was obtained with the corresponding *trans* compounds. About 1 mg. of the substance is dissolved in 1 ml. of glacial acetic acid, and after the addition of 1 drop of conc. sulphuric acid the solution is warmed for a few seconds. It is then cooled and a 1 per cent. solution of bromine in acetic acid is added slowly, drop by drop. A blue to violet colour is usually produced, but this is destroyed by excess of bromine. Thus when only a small amount of material is available, as little as 10γ may often be detected if a very dilute solution of bromine is used. Instead of bromine, acetic anhydride can be employed with advantage, as an excess of that reagent is quite harmless. The formation of the colour depends on the dehydration of the carbinol, and these conditions, whilst adequate to remove the elements of water

from *cis* compounds, are not sufficiently drastic to affect the *trans* compounds. The latter give colours, however, by the following procedure. About 1 mg. of the substance is dissolved in a mixture of 1 volume of phosphorus oxychloride and 3 volumes of quinoline, and the solution is warmed. After cooling, 1 to 2 ml. of glacial acetic acid and 2 to 3 drops of conc. sulphuric acid are added, and then a 1 per cent. solution of bromine in acetic acid, drop by drop. Under these conditions *trans* compounds give the same colour as the corresponding *cis* isomers give under the conditions employed in the first reaction. Thus a substance that gives a colour by the second reaction, but not by the first, almost certainly contains a 17-*trans* hydroxyl group.

A re-investigation of the Liebermann–Burchard reaction showed that this has a close connection with the new colour reaction. Thus blue to green colours are obtained with steroids that possess a *cis* hydroxyl group as well as with true sterols. The Liebermann–Burchard reaction is, however, much less characteristic than the new test and cannot be used to detect the presence and configuration of a 17-hydroxyl group. When androstan-17-ol was treated with potassium bisulphate or copper sulphate, a substance called pseudo-androstene was produced, which has been shown to have the following structure:



This, on treatment with a bromine solution in acetic acid containing a small amount of hydrobromic acid, gives a brown uncrystallisable resin, which dissolves in acetic acid to give a blue solution. This resin is probably a high-molecular, unsaturated compound, and is the prototype of the coloured substance produced in this reaction. F. A. R.

Colour Reactions of Steroids in Relation to Constitution. G. Woker and I. Antener. (*Helv. Chim. Acta*, 1939, **22**, 666–672.)—Digitoxigenin and gitoxigenin give characteristic colour reactions with furfural and conc. sulphuric acid. Thus when to a mixture of 1 ml. of a 0·25 per cent. alcoholic solution of digitoxigenin, 1 ml. of alcohol and 0·05 ml. of a 1 per cent. alcoholic solution of furfural is carefully treated with conc. sulphuric acid so that this forms an under-layer, a narrow, greenish-yellow, weakly fluorescent ring and a narrow orange-coloured ring are produced. These are due to the action of the sulphuric acid on the genin. At the same time a characteristic appearance of the contact-surface is produced; one-half is coloured dark violet, whilst the other half is occupied by lilac-coloured streaks. This effect is produced by the action of furfural and acid on the digitoxigenin, and is also characteristic of isoandrosterone. When 0·1 ml. of furfural solution is used instead of 0·05 ml., the contact-surface appears uniformly dark violet in colour with a slight bluish tinge. When a mixture of 1 ml. of alcohol and 1 ml. of the digitoxigenin solution is treated with conc. sulphuric acid, a bright yellow layer is formed at the bottom of the tube, with an orange ring above it, and at the top an olive or grass-green ring

having a pronounced green fluorescence. The addition of sulphuric acid to a solution of gitoxigenin and furfural results in a cherry-red ring at the bottom due to the action of sulphuric acid on the genin, and above this a double ring, black below and olive-green above, due to the action of furfural on the gitoxigenin. This ring increases in thickness when the concentration of furfural is increased. When sulphuric acid is added to gitoxigenin solution only, a cherry-red ring is formed with an orange to bright red ring below it; this increases in thickness and intensity on standing, a pale rose-coloured zone gradually forming below and a narrow greenish-yellow ring above. F. A. R.

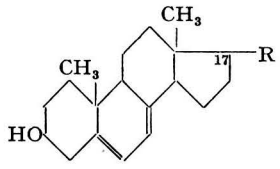
Determination of Vitamin B_1 by means of Yeast. K. Heyns. (*Z. physiol. Chem.*, 1939, **258**, 219–237.)—Six wide-necked 200-ml. bottles are each connected with a gas-burette containing water with a supernatant layer of paraffin oil to prevent solution of carbon dioxide. The bottles are immersed in a thermostat at 30° C., and by means of a suitable device are shaken throughout the experiment. Forty ml. of a medium consisting of a phosphate buffer solution (50 g. of $NH_4H_2PO_4$ and 30 g. of $(NH_4)_2HPO_4$ in 1 litre), containing glucose and salts, are measured into each flask, and the solution to be tested (after dilution to 40 ml.) is introduced; for the blank test, 40 ml. of water are used. The air in the entire apparatus is then displaced with carbon dioxide, and finally 20 ml. of a suspension containing 1 g. of pressed yeast are put into each flask, and the shaking device is set in motion. After a few minutes the reading of each burette is noted, and fermentation is allowed to continue for 6 hours, after which readings are again taken. The amount of carbon dioxide produced in 6 hours is greater in solutions containing aneurin than in the blank solution containing none, and the difference is proportional to the amount of aneurin present, all the other conditions of the experiment being kept constant. When aneurin in an unknown solution is to be assayed, one vessel is used for the blank test, another contains the solution to be tested, whilst the other four contain respectively 1, 5, 10 and 25γ (or other suitable amounts) of aneurin. By comparing the increase in the volume of carbon dioxide produced by the unknown solution with the increase given by the four standard solutions, the aneurin-content of the unknown solution is readily determined. The results were found to be reproducible with a fair degree of accuracy. The addition of aneurin did not bring about any regular increase in the dry weight of the yeast, nor an increase in the volume occupied by the yeast cells, but it did produce an increase in the number of yeast cells per ml. of solution. The absolute amount of carbon dioxide produced was dependent on the amount of yeast taken and on the amount of nicotinic acid present in solution, but neither of these two factors affected the amount of gas formed relative to the blank, nor, in consequence, the validity of the assay. The amount of glucose present was immaterial. Different results were obtained with a fresh and an old yeast, the latter being much more sensitive to aneurin than the former; the use of fresh cultures is therefore very important. The results were not invalidated by the presence of either acetaldehyde or pyruvic acid. Only three substances were found that stimulated carbon dioxide production under the conditions employed in the test. These were aneurin itself, co-carboxylase (1·4γ

was equivalent to 1γ of aneurin) and the pyrimidine component of aneurin. Nicotinic acid, nicotinamide, adermin (vitamin B_6), indoyl acetic acid, inositol and sulphanilamide, were all tested and found to be inactive. It was not found possible to detect less than 0·1γ of aneurin. Similar results were obtained for the co-carboxylase-content of a solution by the fermentation test and by the modified thiochrome test of Westenbrink and Goudsmit (*Acta Neerland. Physiol.*, 1938, 168), and the new method was successfully applied to the determination of aneurin and co-carboxylase in whole blood and blood extracts, flour and flour extract, and extracts of yeast, liver, kidney and other tissues. F. A. R.

Occurrence of Vitamin B_2 (Lactoflavin). Part III. Vitamin B_2 in Yeast and Yeast Extracts. J. Schormüller. (*Z. Unters. Lebensm.*, 1939, **77**, 459–466.)—The material used almost exclusively for the manufacture of yeast preparations is bottom yeast (*Saccharomyces cerevisiae*) from beer fermentation. The lactoflavin-content of a number of samples of commercial yeast and yeast preparations was investigated by extraction with aqueous acetone and colorimetric determination of the lumiflavin produced by irradiation of the extract according to the method already described (*Z. Unters. Lebensm.*, 1939, **77**, 1; Abst., ANALYST, 1939, 215). The results previously found by other workers indicated a range of 1·8 to 3·0 mg. of lactoflavin per 100 g. of dried substance, and the present experiments increased the upper limit of this range to about 4 mg. per 100 g. The experiments show that the process of manufacture of these preparations does not cause any loss of the vitamin activity of the yeast, and confirm the statement of Bing and Remp (*Proc. Soc. Exp. Biol. Med.*, 1934, **31**, 624), *viz.* that the vitamin-content of yeast is not affected by heating at 105° C. for 4 days, although its activity is destroyed by heating at 150° C. for 2 weeks. The results obtained by other workers from animal feeding experiments when calculated to lactoflavin-content (8γ of flavin being assumed equivalent to one rat unit) give figures from 4 to 5 times as high as those obtained by chemical methods, but, since it has been shown that the growth-promoting activity of lactoflavin is increased when the vitamin is associated with the other factors of the vitamin B complex, this discrepancy is to be expected. Experiments with yeast extracts gave figures varying from 4·01 to 6·96 mg. of lactoflavin per 100 g. of dried material, thus proving that, in the manufacture of extracts, treatment of yeast with superheated water under pressure causes no loss of vitamin B_2. As was found previously for liver (*Z. Unters. Lebensm.*, 1939, **77**, 346; Abst., ANALYST, 1939, 445), autolytic changes in yeast cause no measurable decrease in its vitamin-content (*cf.* Pett, *Biochem. J.*, 1935, **29**, 937). The following are examples of the amounts of lactoflavin (mg. per 100 g. of dried substance) found in medicinal preparations of yeast and yeast extract:—a lactoflavin glutathione product, 0·303; a concentrated vitamin B_1 preparation containing vitamin B_2 supplied in ampoules, 0·09; the same preparation in tablet form, 0·840; arsenical tablets containing yeast and yeast extract, 2·095; gastric tablets, 3·075; a liquid vitamin sedative preparation, 0·603. According to Euler and Adler (*Ark. Kem. Min. Geol.*, 1934, **11B**, No. 28; *Z. physiol. Chem.*, 1934, **226**, 88) in dried bottom yeast about 90 per cent. of the lactoflavin is in combination with colloid carriers. In order to determine how much of the flavin of yeast

extracts is so combined, the sample (10 g.) was dissolved in 200 ml. of water and dialysed through cellophane into distilled water at 25° C. and at 3° C. It was found that in yeast extracts about 25 per cent., and in a yeast and malt extract about 35 per cent., of the total lactoflavin is not dialysable. The same result was obtained when the sample was first heated, with or without acidification, before dialysis, and it is concluded that the fraction of lactoflavin not passing through the membrane is not combined with a specific carrier as yellow enzyme, but is adsorbed by the high molecular constituents of the extract. This is confirmed by the fact that a large increase in the time of dialysis reduces the fraction retained by the membrane. An experiment in which a solution of pure lactoflavin was shaken with sufficient glycogen to form a solid glycogen phase showed that 9 per cent. of the flavin was adsorbed by the glycogen, and experiments with yeast gum as the adsorbing substance gave similar results. As both these substances occur in yeast extracts, they are probably the adsorbing agents. To determine the ratio of unesterified lactoflavin to lactoflavin-phosphoric acid in yeast extracts, a pure concentrate was prepared by adsorption upon and elution from fuller's earth and franconite (György *et al.*, *Z. physiol. Chem.*, **1934**, **223**, 27). Aliquot portions of this concentrate were extracted with benzyl alcohol (Emmerie, *Nature*, **1938**, **141**, 416; Euler and Adler, *ibid.*, 790) at *p*H 5·5 and in presence of 3 per cent. of sodium chloride, under which conditions only the unesterified flavin is extracted (Greene and Black, *J. Amer. Chem. Soc.*, **1937**, **59**, 1820), and it was found that 31·94 per cent. of the total flavin was in the free state. Since about 90 per cent. of the total lactoflavin of yeast is in the combined form, a portion of the lactoflavin-phosphoric acid is decomposed in the preparation of the extract. From a consideration of the results obtained it appears that about 70 to 100 g. of beer yeast, 50 to 80 g. of "vitamin yeast" or 40 to 50 g. of yeast extract suffice for the daily need of the human organism. The results of biological experiments indicate that about one-quarter of these amounts is sufficient. A. O. J.

Action of Light on Substances related to Ergosterol. G. A. D. Haslewood. (*Biochem. J.*, 1939, **33**, 454–456.)—3-Hydroxy-Δ5:7-choladienic acid [R = — $CH(CH_3).CH_2.CH_2.COOH$] and its ammonium salt and 7-dehydrostigmasterol [R = — $CH(CH_3).CH:CH.CH(C_2H_5).CH(CH_3)_2$]



were irradiated, and the products were tested for vitamin D activity on rats in comparison with ergosterol [R = — $CH(CH_3).CH:CH.CH(CH_3).CH(CH_3)_2$] irradiated under exactly the same conditions. Whereas the latter contained about 4000 international units of vitamin D per mg., the other substances contained less than 10 units per mg., if any. F. A. R.

Bacteriological

Determination of Hydrogen Peroxide in Bacterial Cultures. E. R. Main and L. E. Shinn. (*J. Biol. Chem.*, 1939, **128**, 417–423.)—Samples of the culture, each measuring 1·0 ml., are placed in small tubes of equal diameter. To each are added 6 drops of potato extract, prepared as described below, and, immediately afterwards, 2 drops of a 10 per cent. solution of *o*-tolidine in glacial acetic acid. The tubes are allowed to stand until the maximum colour has developed. The time required for this is determined for each batch of potato extract by making comparisons with known amounts of peroxide over periods of about 8 minutes; it is usually 4 to 6 minutes. After standing for the necessary length of time the colours of the tubes are compared with those of appropriate standards in a comparator block, a blank, containing only sterile broth, potato extract and *o*-tolidine, being placed behind the standard solution to compensate for turbidity. The peroxide-content is calculated by reference to calibration curves made on the same day as the analyses. The potato extract is prepared from 120 g. of freshly ground potato by allowing it to stand overnight in an ice-box in about 130 ml. of a solution containing equal volumes of glycerin and phosphate buffer *p*H 7·2, the mixture being covered with a layer of mineral oil. The solution is decanted from the solid and, after being centrifuged, is ready for use. It retains its peroxidase activity for several days if a layer of mineral oil is floated on the surface. On the day it is to be used each extract is standardised with known amounts of peroxide. The permanent standards used for measuring the colour are made by suitably diluting a 1 per cent. aqueous solution of malachite green oxalate. When sealed in small tubes they keep indefinitely. Concentrations of 6 to 30γ of hydrogen peroxide per ml. can be estimated with an accuracy of 85 per cent. or more. F. A. R.

Agricultural

Natural Decomposition of Bat Manure: J. Vorster Nel. (*Sci. Bull.* 155, *Dept. Agr. and Forestry*, S. Africa). The composition of so-called fresh bat manure varies very considerably, particularly with regard to the phosphorus-content, owing to contamination by lower and more decomposed layers. The phosphoric oxide content of fresh bat manure as collected from caves is usually higher than the original content, since the manure has a very low apparent sp.gr. so that even slight contamination from lower layers richer in P_2O_5 will increase the percentage considerably, but will not decrease the nitrogen-content proportionately. A sample of fairly fresh manure was divided into two parts, and from one, unbroken droppings were sorted out, (*a*), and the rest was discarded, while the other half, (*b*), was roughly screened to separate most of the stones. After grinding and sieving analysis showed (on the dry samples) total nitrogen (*a*) 10·20, (*b*) 8·12; total P_2O_5 (*a*) 4·15, (*b*) 7·15. The moisture-content of fresh bat manure in the air-dry condition is 7 to 8 per cent., and in caves under dry conditions about 11 to 14 per cent. The fresh manure contains about 10 per cent. of nitrogen on the moisture-free material, and 1 to 2 per cent. of P_2O_5, but accumulation, mostly as tricalcium

phosphate, may bring the phosphate figure in time up to 40 per cent., although the percentage availability of P_2O_5 (2 per cent. citric solution) is higher in fresh samples. As ammonia is one of the first products of decomposition, and is followed by formation of nitrate, the older, more decomposed manure contains a large proportion of nitrate nitrogen. Nitrogen is lost either through drainage, as free ammonia or as free nitrogen, and the manure contains a very active microflora, causing rapid decomposition of organic matter. Decomposition may be so complete that even the less easily soluble ultimate mineral products, such as tricalcium phosphate, are removed, leaving a residue of silica, iron oxide and alumina, contaminated by sand and dust from the cave. The fresh manure contains enough organic matter with reducing properties to reduce all nitrates present to ammonia when digested with sulphuric acid for the determination of nitrogen by the Kjeldahl process. In old manure preliminary reduction of nitrate is necessary. Leaching accounts for the low proportion of sodium and potassium, the K_2O rarely exceeding 1 or 2 per cent. The original sulphur and calcium contents are about 1 and 0·25 per cent. respectively, but gypsum accumulates rapidly owing to external contamination with calcium. D. G. H.

Quantitative Extraction of Carotene from Grass. F. E. Moon. (*J. Agr. Sci.*, 1939, **29**, 295–301.)—Carotene in green vegetable material has usually been determined by a preliminary extraction of total carotenoids followed by the phase separation of carotene and xanthophyll. It is proposed to replace this by direct extraction with petroleum spirit in presence of alcohol. Hot alcoholic potash should not be used for the disintegration of leaf material and saponification of chlorophyll, as the resinous matter which is precipitated hinders subsequent extraction, but the saponification and extraction must be separate; saponification followed by extraction is considered preferable to the reverse order. Five g. of finely-chopped fresh grass, or 2 g. of dried grass meal, are boiled in a flask with reflux condenser for 1½ hours with 40 ml. of 20 per cent. potassium hydroxide solution, and the mixture is cooled and filtered, under reduced pressure, through moistened cotton-wool on a perforated disc. The residue is washed in a beaker four times with 25-ml. portions of industrial spirit and then with petroleum spirit (usually once) until this remains free from colour, all washings being poured through the filter. The precipitation which occurs through the interaction of the alkaline and alcoholic extracts is completed by shaking, and the precipitate is filtered off under reduced pressure and washed with petroleum spirit. The two extracts are shaken well together in a separating funnel; after separation into two layers the alcoholic solution is re-extracted three times with petroleum spirit. If the colour of the final extract is completely removed by 92 per cent. methyl alcohol the extraction of carotene is complete; otherwise, further extraction is required. After the combined petroleum spirit solutions have been repeatedly extracted with 92 per cent. methyl alcohol until this remains colour-free, the solution of carotene in petroleum spirit is washed with water and the pigment present may then be determined colorimetrically or otherwise. Results obtained by this method have been compared with those given by the methods of Ferguson and Bishop (ANALYST, **1936, 61**, 515) and Pyke (*J. Soc. Chem. Ind.*, **1936, 55**, 139T; Abst., ANALYST, **1936,**

61, 497), the Lovibond tintometer and Ferguson's curve (ANALYST, 1935, **60**, 680) being used. The variance and standard error have been shown to be smaller than those for the other two methods, and it has been found that the results obtained are reproducible with considerable accuracy. E. B. D.

Substitution of Lactic Acid for Citric Acid in the Analysis of Basic Slag. A. Heiduschka and H. Wünsche. (*Chem.-Ztg.*, 1939, **63**, 317.)—In the course of experiments with a slag having a total phosphate-content equivalent to 13·2 per cent. of P_2O_5, Sirot and Joret have shown that 10·8 to 10·9 der cent. can be dissolved out in 30 minutes by means of a 2 per cent. solution of citric acid or by solutions of equivalent molecular strengths of lactic acid or malic acid; 7·4 to 7·6 per cent. by acetic acid or tartaric acid; 3·2 per cent. by oxalic acid, all of equimolecular strengths (*cf.* ANALYST, 1919, **44**, 180). Since citric acid and lactic acid behaved similarly and since the former was then the cheaper, these results have hitherto attracted little interest. The present authors have now repeated the experiments with 1, 2 and 3 per cent. solutions of these two acids and have shown that the difference between the two sets of results falls within the permissible limits of experimental error (*i.e.* 0·3 per cent., as phosphoric acid). This applied whether the period of agitation during extraction was 60 minutes with the 1 per cent. solution or 30 to 120 minutes with the 2 per cent. solution. Comparisons made on four samples of basic meal and with 2 per cent. solutions of the acids showed that the average difference between the results obtained with the two acids was 0·20 and 0·28 per cent. for the Lorentz and Popp (iron citrate) methods, respectively.

J. G.

Water

Analyses of Water of the Old Sulphur Well at Harrogate. (*The Harrogate Spa Medical J.*, 1939, **2**, 16.)—The Spa authorities are exhibiting in the Royal Pump Room a chart which shows in graphical form the saline and sulphur contents of the Old Sulphur Water as determined by various analysts from 1783 to the present time. The chart affords convincing evidence of the constancy of composition of this famous spring.

	Calculated in parts per 100,000	
	Total solids	Sulphur
Walker (1783)	1587	—
West (1823)	1464	—
Hunter (1830)	1451·4	—
Hofmann (1853)	1565·6	9·08
Muspratt (1867)	1584	9·62
Davis (1872)	1495·1	9·16
Thorpe (1875)	1495·9	9·33
Lowson (1912)	1508·5	9·43
Present day	1553·0	9·73

Organic

Separation of Oleic Acid from Saturated Acids and Linolic Acid, with Observations on the Preparation of Oleic Acid. P. J. Hartsuch. (*J. Amer. Chem. Soc.*, 1939, **61**, 1142–1144.)—A comparative study of various methods for the separation of saturated from unsaturated fatty acids showed that the Brown and Shinowara method (*J. Amer. Chem. Soc.*, 1937, **59**, 6; Abst., ANALYST, 1937, **62**, 325) was the most efficient. The separation of oleic from linolic acid was eventually made by an initial crystallisation of oleic acid from 10 vols. of acetone at −60° C. Owing to the separation of a considerable amount of linolic acid only 5 vols. of acetone were used for the last three crystallisations, which were carried out at −40° C. This gave a 10 per cent. increase in yield of precipitate without decreasing the effectiveness of the separation of the residual linolic acid. Still more of the remaining saturated acids were removed by a lead soap precipitation in 95 per cent. alcohol, with the use of three times as much lead acetate as would be required to react with the estimated amount of acids present. Distillation at about 1 mm. pressure in a still with electrically heated glass column 4 feet high, and with the still-head temperature controlled to give a reflux ratio between 5 : 1 and 10 : 1 was then carried out, and the 6th fraction was found to contain 97·8 per cent. of oleic acid, 1·0 of linolic and 1·2 per cent. of saturated acids. This is taken as probably representing the maximum purity obtainable by present methods.

D. G. H.

Elaidinisation of Linolic Acid. J. P. Kass and G. O. Burr. (*J. Amer. Chem. Soc.*, 1939, **61**, 1062–1066.)—Elaidinisation of linolic acid was carried out with nitrogen oxides, and most successfully with Poutet's solution, and with selenium. The solid crystalline product isolated had m.p. 28–29° C.; iodine value (Wijs), 178·3; thiocyanogen value, 90·0; neutralisation equivalent, 280. It gave a crystalline lead salt and was spectroscopically inactive. The calculated values for the formula $C_{18}H_{32}O_2$, are carbon 77·06, and hydrogen 11·50, and the figures found were 76·89 and 11·25, respectively. Equal parts of a liquid and solid tetrabromide (m.p. 78° C.) were found on bromination of the crystalline linolelaidic acid dissolved in ligroin. Partial alkaline oxidation with potassium permanganate resulted in the production of two sativic acids, with m.p. 146 and 122° C., respectively. In addition to the solid acid a liquid linolelaidic acid was produced in an impure form, together with conjugated by-products. Only liquid bromides could be obtained from this acid and partial oxidation again produced two sativic acids, with m.p. 126–127 and 156–158° C., respectively. It is suggested that linolic acid is either totally converted on elaidinisation or is present in the reaction mixture in too small quantities to be detected by bromination or partial oxidation, the removal of the solid isomer and conjugated by-products leaving a liquid fraction characterised by its ligroin-soluble bromide. D. G. H.

Use of Selenium in Kjeldahl Digestion of Leather for Nitrogen Determination. D. Williams. (*J. Amer. Leather Chem. Assoc.*, 1939, **34**, 261–263.)—The nitrogen in 30 samples of leathers of varying composition (from loaded to unloaded) was determined. Analyses were made in duplicate by the Official

A.L.C.A. method, and by that method with the addition of approximately 0·3 g. of selenium. Closer agreement between the duplicate determinations was obtained in the selenium digestions, and there was a saving of time in the digestion, only 1 hour being required instead of the usual 2¼ hours. Averages of all the determinations were as follows:—by the Official method: Nitrogen, 6·069, or as hide substance, 34·10 per cent., and by the modified method, 6·085, or as hide substance, 34·20 per cent. D. G. H.

Inorganic

Tetraphenylarsonium Chloride as an Analytical Reagent. Determination of Mercury, Tin, Cadmium and Zinc. H. H. Willard and G. M. Smith. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 269–274.)—*Mercury.*—The method depends on the precipitation of a white crystalline compound, which is insoluble in presence of sodium chloride, in accordance with the reaction

$$2(C_6H_5)_4As^{\cdot} + HgCl_4'' \rightarrow [(C_6H_5)_4As]_2HgCl_4.$$

The unused excess of the reagent is determined volumetrically; gravimetric determination is not possible because no suitable washing liquid could be found. The solution of mercury as mercuric nitrate is neutralised, if necessary, with sodium hydroxide solution and rendered slightly acid with hydrochloric acid. A limited excess of standard (0·01 to 0·03 *M*) tetraphenylarsonium chloride reagent is added, with constant stirring, and then enough sodium chloride to give a concentration of 1 to 2·5 *M* sodium chloride. The volume of the liquid should be 60 to 120 ml., depending on the quantity of mercury present. The precipitate is filtered off after 15 to 60 minutes and washed several times with saturated sodium chloride solution, and the filtrate and washings are titrated potentiometrically with standard iodine as previously described (*id.*, 1939, **11**, 186; Abst., ANALYST, 1939, 452); 1 ml. of 0·01 *M* reagent ≡ 1·0031 mg. of mercury. The error with amounts of mercury from 0·5 to 107 mg. in pure solutions is about ±0·06 mg. With the smaller amounts, a considerable excess of reagent is required and some hours are necessary for complete precipitation. Copper interferes, not with the precipitation, but with the titration of the filtrate, by liberating iodine; this may be avoided by adding sodium citrate and citric acid before the titration. In presence of ferric iron (up to 300 mg.) it is necessary to add sufficient syrupy phosphoric acid to discharge the yellow colour before precipitating the mercury, and then, prior to the titration, to add a further similar amount of phosphoric acid plus twice the weight of disodium phosphate. The interference of manganese and stannic ions can be overcome by the addition of tartrate to the solution before precipitation, but that of cadmium, zinc and bismuth, cannot be eliminated. Other interfering ions are tungstate, chromate, molybdate, chlorate, iodide, bromide, fluoride and thiocyanate.

Tin.—The method is similar to that for mercury. The compound precipitated is $[(C_6H_5)_4As]_2SnCl_6$. At the stage of precipitation the volume of solution should be 60 ml.; the concentration of sodium chloride 2·5 to 3 *M* for quantities of tin up to 30 mg., or 1·5 to 2 *M* for larger quantities; the acid concentration 0·4 to 1 *M*; 1 ml. of 0·01 *M* reagent ≡ 0·5935 mg. of tin. Phosphate, citrate, oxalate and fluoride prevent precipitation of tin; tartrate interferes only very slightly. The

method is also affected by those substances which interfere with the determination of mercury.

Cadmium and Zinc.—These may be determined by methods similar to that for mercury. The compounds precipitated are also of analogous composition. The precipitation is quantitative in presence of a fairly high concentration of sodium chloride. The chlorocadmiate precipitate is considerably more soluble than the chloromercuriate compound, and the chlorozincate compound is still more soluble. Owing to the rather high solubility, these determinations are in general subject to more interferences than the others described above. S. G. C.

Separation and Determination of Copper and Nickel by Salicylaldoxime. L. P. Biefeld and D. E. Howe. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 251–253.)—Tests of the salicylaldoxime method showed that copper may be separated from nickel by precipitation with the reagent at *p*H 2·6–3·1. After precipitation of the copper, nickel may be quantitatively thrown down by adjusting the *p*H to 7·0–9·9. There was slight co-precipitation of nickel with the copper; thus with 500 mg. of nickel in the solution (250 ml.) only about 0·5 mg. of the nickel complex, $Ni(C_7H_6O_2N)_2$, was occluded when 25 mg. of copper were precipitated. The co-precipitation of iron with the copper was about 10 times as much as that of nickel. S. G. C.

Separation and Determination of Aluminium and Beryllium with Tannin. M. L. Nichols and J. M. Schempf. (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 278–280). Moser and Niessner's method has been studied with regard to regulation of *p*H value, time of digestion and so on. The following process is recommended:—To the solution containing not more than 0·08 g. of alumina and beryllia together, 25 ml. of saturated ammonium acetate solution are added, and the *p*H is adjusted to 4·6 by the use of 6 *N* sulphuric acid and 1 : 1 ammonia. The solution is heated to boiling, tannin is added slowly (50 ml. of a 3 per cent. solution or at least 12 to 15 times the combined weight of alumina and beryllia present), and the liquid is digested on a steam-bath for 1 hour. After cooling to room temperature, the aluminium-tannin precipitate is filtered off on a coarse-textured paper, washed with a 5 per cent. solution of ammonium acetate containing a little tannin and adjusted to *p*H 4·6, ashed, ignited at 1200° C., and weighed. Beryllium is determined in the filtrate as in the method of Moser and Singer (*Monatsh.*, 1927, **48**, 673; ANALYST, 1928, **53**, 402). Good results were obtained in tests on mixtures. S. G. C.

Quantitative Determination of Calcium by means of Loretin. N. Schoorl. (*Pharm. Weekblad*, 1939, **76**, 620–625.)—Loretin (7-iodo-3-hydroxy quinoline-5-sulphonic acid) has been used as a qualitative reagent for calcium (*cf.* Van Zijp, ANALYST, 1932, **57**, 801), and under the following conditions it may be used quantitatively:—To 2·5 ml. of the solution to be tested (which should contain not more than 2 per cent. of calcium) are added 2·5 ml. of a buffer solution (*p*H 4) prepared by dissolving 80 ml. of 30 per cent. acetic acid and 10 g. of crystalline sodium acetate in water and diluting to 100 ml. The beaker is warmed on a water-bath, 25 ml. of the boiling reagent (see below) are introduced, and the mixture is shaken and left on the water-bath for 15 minutes. It is then allowed to cool slowly (important), and,

with small quantities (0·5 to 10 mg.) of calcium it is advisable to allow the cooling to take place in a closed box. The orange-red crystalline precipitate of monocalcium loretinate is separated on a sintered glass crucible (size 3G3), and washed in succession twice with the reagent, twice with 96 per cent. alcohol and once with acetone, 3 ml. of liquid being used for each wash (see below); the crystals are large and coarse, and are easily transferred to the filter. They are dried in air, and finally in a vacuum desiccator until constant in weight; they then have the composition $3[Ca(C_9H_4N.I.OH.SO_3)_2],10H_2O$, and the weight of calcium is obtained by dividing the weight of the precipitate by 20. This factor applies only when 10 to 40 mg. of calcium are present. With 4 to 16, 2 to 8 or 0·5 to 3 mg. of calcium, the volumes of original solution that should be used are 1·0, 0·5 and 0·2 ml.; buffer solution, 1·0, 1·5 and 0·2 ml.; reagent, 10, 5 and 2 ml., respectively. The corresponding weights of precipitate are then 80 to 320, 40 to 160 and 10 to 60 mg., respectively, and the appropriate factors are calculated accordingly. The reagent is prepared by shaking 8·8 g. of loretin with 200 ml. of water and adding 6·5 ml. of 4 *N* sodium hydroxide solution. The red-brown solution of sodium loretinate ("Meditren") is diluted to 250 ml. and filtered; if, in the course of time, fine crystals are deposited from the filtrate, the presence of traces of calcium as an impurity is indicated, and the solution should again be filtered. Metals other than sodium, potassium or magnesium must be absent. The precipitate, which is stable in air, may also be determined volumetrically by de Jong's method (*Pharm. Weekblad*, **1937**, **74**, 608), each ml. of 0·1 *N* silver nitrate solution used being equivalent to 2 mg. of calcium. An important feature of the method is the amount and nature of the solvents used for washing the precipitate; thus, the solubilities of the precipitate in water, 50 and 96 per cent. alcohol, acetone and ether are 1 part in 1250, 800, 20,000, 50,000, and more than 200,000, respectively, at room-temperature.

J. G.

Catalytic Action of Selenium in the Kjeldahl Digestion Method. A. Sreenivasan and V. Sadasivan. (*Z. anal. Chem.*, **1939**, **116**, 244–252.)—The mechanism of the catalytic action of selenium under the conditions of the Kjeldahl process was elucidated. By using selenium, selenious acid, and selenic acid, the authors have proved that selenious acid is the stable compound in hot strong sulphuric acid. If, however, mercuric oxide is present, selenious acid is rapidly and completely converted into selenic acid (under those conditions the stable form), and at a greater speed than that of the reduction of selenic into selenious acid by the organic matter. This explains the speed with which organic matter is destroyed by sulphuric acid in presence of mercuric oxide and selenium, the process requiring only one-third of the time taken when mercuric oxide is omitted. The form in which the selenium is added makes no difference. If no mercuric oxide is used, the selenium is wholly present as selenious acid at the conclusion of the operation; otherwise, only selenic acid will be found. It is concluded that the catalytic action of selenium in presence of mercuric oxide is due to the reaction $Se \rightarrow SeO_2 \rightleftharpoons SeO_3$. Without mercuric oxide the reversible reaction $Se \rightleftharpoons SeO_2$ occurs, resulting in more sluggish oxidation. The catalytic action of selenium is greatest during the initial stages of the digestion. W. R. S.

Determination of Fluorides in Wood Preservation Technology. I. Analysis of Solutions of Fluorides in Water. B. Ikert. (*Chem.-Ztg.*, 1939, **63**, 324.)—Existing methods (*e.g.* precipitation as calcium fluoride or titration with ferric chloride) are unsuitable, being too slow and easily upset by other substances (*e.g.* chromates, arsenates and dinitrophenol) likely to be present in the solutions concerned. The following method, which can be carried out in 35 to 40 minutes, is recommended when either a chromate or arsenate is present or when both are absent:—A mixture of 25 ml. of the solution to be tested (containing 0·1 to 0·25 g. of sodium fluoride), 5 ml. of a 3 per cent. solution of sodium silicate and 2 drops of methyl orange solution is neutralised with 10 per cent. hydrochloric acid. Two drops of the acid are then added in excess, and are followed by 1 g. of solid potassium chloride, and when this has dissolved 35 ml. of alcohol are added. After 20 minutes the precipitate of potassium fluosilicate is separated by filtration on a filter-paper or an asbestos-lined Gooch crucible, and washed with a 2 per cent. solution of potassium chloride in 50 per cent. alcohol until the total filtrate amounts to 100 ml.; filtration is more rapid on the Gooch crucible. The contents of the filter are then washed back into the original containing-vessel with 100 ml. of hot water, and the mixture is titrated with 0·1 *N* sodium hydroxide solution with phenolphthalein as indicator until a weak red colour is obtained; 0·3 ml. is added to the reading to allow for losses due to solubility of the precipitate. When approximately 0·2 g. of sodium fluoride was taken the resulting errors were +0·4, +0·8, and 0 to +0·3 in presence of 0·6 g. of potassium dichromate, 0·4 g. of sodium arsenate, and in absence of both salts, respectively. If chromates and arsenates are present together, they may combine under the influence of the alcohol to form a compound containing tervalent chromium, which is sparingly soluble and uses up some of the sodium hydroxide solution. The formation of this compound is accelerated by standing, by a high *p*H value, and by exposure to light, and the following precautions must therefore be taken. After neutralisation to methyl orange 0·3 ml. of the acid is added in excess, and, 15 minutes after the addition of the alcohol, another 0·3 ml. is added. The mixture is then immediately filtered. This procedure delays precipitation of the chromium compound, especially if exposure to sunlight is avoided. Duplicate tests on a solution containing 0·1357 g. of sodium fluoride, 0·125 g. of sodium arsenate, 0·1875 g. of potassium dichromate and 0·05 g. of dinitrophenol (*i.e.* corresponding with a commercial solution used for the impregnation of wood) gave results having an error of +0·2 and +0·4 per cent. If, however, large quantities of arsenates or chromates, or both, are present, they may be occluded by the precipitate and the results may be 1 per cent. high. J. G.

New Specific Reaction for Nitric Acid and Nitrates. M. M. Pesez. (*J. Pharm. Chim.*, 1939, **29**, 460–465.)—With the exception of a few depending upon the formation of insoluble nitrates, the reactions used for the detection of nitrates are colour reactions which, although very sensitive are not specific, since they depend upon the oxidising action of the nitrate ion. The reaction of Janowski (*Ber.*, 1891, 971), in which *m*-dinitrobenzene is condensed with acetone in presence of alkali to form an intense violet colour, is adapted to the detection of nitric acid

and its salts and, since the basis of the reaction is the formation of a nitro-compound, it is specific for the nitric ion. A few particles of the substance to be tested are treated in a test-tube with 12 drops of conc. sulphuric acid and 2 drops of benzene and, after thorough agitation, the tube is heated in a boiling water-bath for 3 minutes. If the substance to be tested is a liquid it is neutralised, evaporated to dryness in a glass capsule, and treated with sulphuric acid and benzene, the mixture being stirred well with a glass rod before being heated on the water-bath. When cold the reaction mixture is treated with 5 to 8 ml. of acetone followed by sufficient sodium hydroxide solution (about 20 per cent.) to render the mixture alkaline. If nitrate is present in the original substance an intense violet colour is formed. The colour changes very slowly to reddish-violet and finally to blood-red, the change being accelerated by the presence of water. If the nitration mixture is heated over a free flame instead of on the water-bath, trinitrobenzene may be formed, and the colour will then vary from reddish-violet to red. With the procedure described the test will detect 0·5 mg. of potassium nitrate in 1 ml. of solution. In presence of bromide or iodide the acetone is coloured by the liberated halogen, but the colour disappears when the sodium hydroxide is added, and the presence of these substances does not interfere with the test. Since nitrobenzene gives no colour with acetone and alkali, it may replace benzene in the test; it has the advantages that it is miscible with conc. sulphuric acid and that heating of the reaction mixture is unnecessary. If desired, a 10 per cent. solution of nitrobenzene in conc. sulphuric acid may be used as the reagent, and the mixture is allowed to stand for 2 or 3 minutes before the addition of acetone and alkali. Nitrous acid and nitrites do not answer to the test, and no compound has yet been found which simulates the action of the nitric ion. A. O. J.

Microchemical

Microscopic Examination of Polymorphous Compounds. (I) L. Kofler and E. Lindpaintner, (II) E. Lindpaintner. (*Mikrochem.*, 1938, **24**, 43–58; 1939, **27**, 21–41.)—(I) By using an electrically heated block, the melting point of individual crystals may be readily observed under the microscope. Transformation from one form to another can usually be best observed between crossed nicols. It is frequently possible to obtain individual crystals of the different modifications by micro-sublimation. Nipagin, veronal, atophan, morphine and a number of other substances sublime in different forms. Sublimation is carried out both at normal and reduced pressures. Benzil gives three modifications melting at 95°, 41·5° and 27° C., and 8-hydroxyquinoline gives four melting at 73·5°, 65°, 57–58°, and 38–39·5° C., respectively. Benzidine gives five modifications, four of which melt at 129°, 125°, 120·5–121° and 116·5–117° C., respectively, whilst the m.p. of the fifth cannot be determined. Ten photomicrographs are given. (II) Polymorphism is shown by the following substances:—Phenyl benzoate: three forms, melting at 69°, 56·5° and 51–52° C.; benzoyl-l-ecgonine: four forms, melting at 202–203°, 179–181°, 130–135° and 100–105° C.; quinizarin enantiotropic: two modifications —one orange, melting at 195° C., and the other, red, melting at 201° C.; chrysophanic acid: two forms, melting at 195° and 190° C.; coumarin: three forms,

melting at 68·5°, 64·5° and 55° C.; gallic acid: two forms melting at 258–265° and 225–230° C.; hydroquinone: two forms, both melting at 172·5° C.; morphine hydrochloride: two forms, melting at 295–300° and 280–284° C.; nipagin: six forms, melting at 127°, 116°, 110°, 110°, 109° and 106° C.; *o*-nitrobenzaldehyde: two forms, melting at 42–42·5° and 39° C.; *m*-nitrobenzaldehyde: two forms, melting at 56–57° and about 51° C.; *p*-nitrobenzaldehyde: two forms, melting at 105° and 104–104·5° C.; phenanthraquinone: two forms, melting at 210–211° and 207° C.; veronal: four forms, melting at 190°, 183–190°, 183° and 176° C.; *m*-xylenol: two forms, melting at 62–63° and about 55° C. Details are given of methods of conversion. J. W. M.

Inorganic Spot Tests. F. Feigl. (*Rec. Trav. chim. Pays-Bas*, 1939, **58**, 471–480.)—(1) *Test for tungsten with diphenyline.*—The reagent is preferable to benzidine. A drop of a 1 per cent. solution of diphenyline hydrochloride in 2 *N* hydrochloric acid is added to a drop of the test solution; a turbidity indicates tungsten. For the smallest amounts comparison with the result of a blank test after 15 minutes is necessary; the limit of identification is then 6γ. (2) *Test for calcium with the osazone of dihydroxy tartaric acid.*—The test is not very selective, and salts other than those of ammonium and the alkali metals interfere. A white precipitate, visible against a dark background, is formed from solution as dilute as 1 in 5 million. The test has been found useful to distinguish between tap-water and distilled water. (3) *Test for sodium with zinc uranyl acetate.*—Limit of identification: 12·5γ of sodium on a black spot-plate, or 2·5γ by the greenish-yellow fluorescence produced when a drop of the solution on filter-paper is exposed for 1–4 minutes while still moist, to ultra-violet light. The reagent solution of zinc uranyl acetate is prepared by mixing a solution of 10 g. of uranyl acetate in 6 g. of 30 per cent. acetic acid, diluted with water to 50 ml., with a solution of 30 g. of zinc acetate in 3 g. of 30 per cent. acetic acid, diluted to 50 ml. A trace of sodium chloride is added to the warm mixture and, after 24 hours, any sodium zinc uranyl acetate is filtered off. (4) *Test for hydrazine with salicylaldehyde* (this test may also be applied quantitatively).—When a drop of the reagent is mixed with a drop of the test solution in a micro-test-tube a white precipitate is formed; limit of identification: 0·1γ of hydrazine. The reagent is prepared by boiling 5 g. of salicylaldehyde with 600 ml. of water and 20 ml. of 50 per cent. acetic acid until the oil has disappeared. The solution is filtered before use. (5) *Test for hydroxylamine with salicylaldehyde and copper acetate.*—The solutions must be mixed in the following order: A drop of the reagent solution (as above, Test 4) is mixed in a test-tube with a drop of the test solution and then with 2 drops of a filtered solution of 5 g. of copper acetate in 150 ml. of water and 1 or 2 ml. of 50 per cent. acetic acid. In presence of hydroxylamine a pale yellow precipitate or cloudiness is formed; limit of identification: 1γ. (6) *Test for hydrazoic acid by formation of silver or iron azide.*—A drop of the test solution is placed in a small tube closed with a glass stopper with a knob underneath on which hangs a drop of ferric chloride or silver nitrate solution. The acid is liberated on warming very slightly with 1 or 2 drops of dilute hydrochloric acid and, acting on the ferric chloride or silver nitrate, gives a red colour with the former

or cloudiness with the latter. Limit of identification: 5γ of sodium azide. (7) *Test for ferrocyanide with uranyl acetate.*—A drop of *N* uranyl acetate solution is mixed on a spot-plate with the test solution. A brown precipitate is formed; limit of identification: 1γ. The test may also be carried out on filter-paper. (8) *Test for ferricyanide with benzidine.*—The test may be used in presence of ferrocyanide. The test solution is mixed with a drop of a saturated solution of benzidine in 2 *N* acetic acid; it is advisable to add also a drop of a 1 per cent. lead nitrate solution. A blue colour forms with as little as 5γ of potassium ferricyanide even in the presence of excess of ferrocyanide. (9) *Test for chlorate and periodate by formation of complex manganese phosphate.*—A drop of the test solution is mixed with a drop of a mixture of equal parts of saturated manganese sulphate solution and syrupy phosphoric acid and gently heated in a micro-crucible. A violet colour appears on cooling, in presence of chlorates or periodates. A pale colour may be intensified by adding a drop of a 1 per cent. alcoholic solution of diphenyl carbazide, when the limit of identification is 0·05γ of chlorate and 5·0γ of periodate. (10) *Test for sulphate with barium carbonate and phenolphthalein.*—A drop of the neutral test solution is mixed with a drop of a barium carbonate suspension and evaporated to dryness. On addition of a drop of an alcoholic solution of phenolphthalein a red colour indicates sulphates. It is advisable to ascertain if there is any interference when these tests are applied to the analysis of material containing a number of other ions. J. W. M.

Microcrystalline Reaction for Identifying Anthracene. G. Denigès. (*Bull. Trav. Soc. Pharm., Bordeaux*, 1939, 1; *J. Pharm. Belg.*, 1939, **21**, 308.)—A few particles of the product to be identified are placed on a glass slide and mixed with one drop of a solution of bromine in chloroform (preferably 5 per cent., never more than 10 per cent.). If anthracene is present, characteristic yellow needles of anthracene dibromide (m.p. 221° C.) are formed. The other cyclic hydrocarbons do not give this reaction. E. B. D.

Microcrystalloscopic Identification of Bromine in Chloroform Solution. G. Denigès. (*Bull. Trav. Soc. Pharm., Bordeaux*, 1939, 1; *J. Pharm. Belg.*, 1939, **21**, 308.)—Bromine, if present in aqueous solution, may be identified by the converse of the anthracene reaction (see preceding abstract). The solution is shaken well with chloroform and, after the mixture has stood, 1 ml. of the chloroform layer (which contains all the bromine) is transferred to a small conical flask, care being taken not to remove any water from the upper layer, and 2 droplets of a 1 per cent. solution of anthracene are added. If the solution becomes reddish-yellow, 1 or 2 drops are immediately evaporated and the crystals of anthracene dibromide formed are examined microscopically (×130 to 200); otherwise, the chloroform solution is concentrated before evaporation. E. B. D.

Electrolysis of Gold Solutions. M. G. Raeder and O. S. Kyllingstad. (*Mikrochem.*, 1939, **27**, 112–117.)—Gold chloride solutions containing 0·4 to 30γ of gold may be electrolysed quantitatively if they contain 3 to 5 drops 2 *N* potassium chloride solution. With the Pregl electrolysis apparatus deposition is complete after 20 minutes' boiling with a current density of 20–25 milliamps

per sq.cm.; at room temperature when no stirring is used 4 hours is necessary. As anode a hard pencil lead may be used, but for a quantitative determination the gold is preferably precipitated upon sheets of assay lead, free from gold. The lead is then eliminated by the usual methods, and the residual bead of gold is measured micrometrically. J. W. M.

Physical Methods, Apparatus, etc.

Chelate Compounds as Flotation Reagents. C. C. De Witt and F. Von Batchelder. (*J. Amer. Chem. Soc.*, 1939, **61**, 1247–1249.)—The chelate compounds possess peculiar characteristics which have led to the investigation of their applicability as flotation reagents. Salicylaldoxime has been found effective for the separation of chalcocite, covellite, azurite, malachite and cuprite from siliceous gangue materials. The siliceous gangue material (50 g.) was placed in the flotation cell (enamelled cast-iron type) with 0·5 g. of the ore, 200 ml. of water were added and the stirrer was started (1750 r.p.m.). The aqueous solution of the required strength of oxime was then added, and was followed, after 3 minutes, by 2·5 mg. of the frothing agent (1 vol. of pine oil in 9 vols. of ethanol). The froth was collected over a period of 3 minutes, filtered on a Gooch crucible, dried and weighed, and its copper-content was determined. The residual gangue was also analysed to detect any possible errors in the previous analyses of the ores. Maximum recoveries of copper varied from 89·7 per cent. for cuprite (8 lb. of oxime per ton) to 97·7 per cent. for malachite (2·0 lbs. of oxime). It was found that replacement of the *ortho*-hydroxyl group of salicylaldoxime by a methoxy group gave a methoxylbenzaldoxime which was of no use as a flotation reagent. D. G. H.

Normal Oximes as Flotation Reagents. C. C. De Witt and F. Von Batchelder. (*J. Amer. Chem. Soc.*, 1939, **61**, 1250.)—All the normal oximes, including octyl oxime, were tested qualitatively as flotation reagents, but heptaldoxime and octaldoxime were the only ones that proved suitable for the separation of oxide, carbonate and sulphide ores of copper from siliceous gangue material. The flotation experiments were carried out as before (see above). Maximum recoveries of copper per cent. were as follows for (*a*) *n*-heptaldoxime and (*b*) *n*-octaldoxime:—chalcocite (*a*) 98·7 (0·4 lb. of oxime per ton), (*b*) 97·2 (2 lb.); malachite, (*a*) 89·6 (2 lb.), (*b*) 92·4 (2 lb.); covellite, (*a*) 95·4 (2 lb.), (*b*) 96·5 (2 lb.); azurite, (*a*) 85·5 (2 lb.), (*b*) 87·0 (2 lb.); cuprite, (*a*) 96·8 (4 lb.), and (*b*) 96·4 (2 lb.). D. G. H.

Viscometric Method for the Characterisation of Soluble Starch. W. A. Richardson. (*Chem. and Ind.*, 1939, **58**, 464–465.)—Since "soluble starch" is not an individual substance or even a mixture having constant and reproducible properties, and since the chemical reagents used in its manufacture vary considerably, it is not possible to evaluate such products by chemical methods. As with cellulose, evaluations have been made by viscometric methods (see Gallay and Bell, *Canad. J. Res.*, 1936, **14**B, 360), but difficulty has been experienced in finding a suitable solvent, because the lightly-modified starches swell before

dissolving, and they must then be dispersed by the action of heat, and this results in further modification. Moreover, solutions of such starches in water are unstable and their viscosities are affected by the presence of electrolytes, whilst the viscosities of solutions in alkali do not remain constant, and are sensitive to mechanical treatment and to the presence of impurities. The solvent now recommended is a solution of calcium thiocyanate. A suspension of starch in cold water is poured into sufficient boiling water to produce a 2·5 per cent. solution or paste, which is then heated for about 1 minute and cooled. It is then passed through an homogeniser, and the concentration is determined by means of the dichromate method of Richardson, Higginbotham and Farrow (*J. Text. Inst.*, 1936, **27**, 131T). To 10 ml. of this solution are added 15 ml. of a 50 per cent. solution of calcium thiocyanate, a control solution consisting of 30 g. of calcium thiocyanate in 100 ml. of water being also prepared. The viscosity of 10 ml. of each solution is then measured in separate U-tube viscometers (*cf.* Higginbotham and Richardson, *J. Soc. Chem. Ind.*, 1938, **57**, 234), which are placed in a water-bath at 25° ± 5° C., and if n is the viscosity of the starch solution relative to that of the control solution, and c is the concentration of starch in g. per 100 ml., then the quantity $(\log n)/c$ is known as the "thiocyanate viscosity" (TV). It has been found that with potato, sago and maize starches, which have been modified in solution by means of hot dilute acids and then neutralised, the degree of modification is accurately measured by the reducing power (R) as determined by the method of Richardson, Higginbotham and Farrow (*loc. cit.*); moreover, since for such starches TV is a measure of R, TV may also be used as a satisfactory index of the degree of modification. With other starches (*e.g.* those that have been modified by oxidation, or by treatment in a granular condition with acids, followed by washing, which removes some of the reducing substances), TV is to be preferred to R as an index of the extent of modification. Tabular data show that with unmodified starches and starches that have been lightly modified by acid, the thiocyanate viscosity is very sensitive to mechanical treatment and therefore varies with the efficiency and duration of the homogenising process; since, however, this effect decreases as the degree of modification increases, when R exceeds a certain value (namely 40) homogenisation is unnecessary. Modification by oxidation, however, may result in a lower value of R than modification to the same extent, but by an acid process, and it may not be necessary to homogenise even when R is less than 40; values of R of from 5 to 160 are recorded for commercial soluble starches which have been modified by different methods and to different extents. When c is between 0·6 and 1·0 per cent. TV is independent of c, and so long as c is known accurately it is unnecessary to adjust it to a given value. TV is also independent of the concentration of sodium sulphate (up to 17·5 per cent. on the weight of starch) or of sodium chloride within the range of concentrations such as are likely to occur in commercial soluble starches. TV is slightly lower at 60° C. than at 25° C., but the difference rarely exceeds 5 per cent., and in any event the use of a control solution in the same thermostat eliminates any errors from this source. The thiocyanate solutions of acid-modified starches are unchanged in appearance and TV after 36 days at room-temperature, but the TV of certain oxidised starches falls slowly by about 0·02 in 8 to 36 days. J. G.

Electrolytic Polishing of Tin and its Application to Micrographic Examination. P. A. Jacquet. (*Intl. Tin Res. Development Council*, Pubn. No. 90, Feb., 1939.)—The usual technique for polishing metals for microscopical examination is unreliable for a soft metal such as tin, which is easily deformed by mechanical treatment and shows recrystallisation phenomena even at room temperature (*cf.* Taffs, *id.*, Pubn. No. 47, 1936). Polishing by electrolytic methods, *i.e.* by anodic treatment under controlled conditions (*cf.* Jacquet, *Compt. rend.*, 1935, **201**, 1473; 1936, **202**, 402; 1937, **205**, 1232; Elmore, *Phys. Rev.*, 1938, **53**, 757) is therefore preferable; in addition, this method stresses the crystal boundaries and produces characteristic corrosion figures within the crystals. The electrolytic cell is a beaker (250 or 500 ml.) in which are immersed vertically and a few cm. apart the specimens to be polished (the surface of which should not exceed 10 sq.cm.) and a piece of tinfoil having a surface larger than that of the sample. These form the anode and cathode, respectively, and are connected in series with a source of D.C. (25 to 40 volts), an ammeter, a variable resistance (about 150 ohms) and a switch. The beaker, which is immersed in a vessel of cold water, also contains a stirrer, a thermometer, and a solution prepared by pouring 806 ml. of 98 per cent. acetic anhydride very slowly into 194 ml. of perchloric acid (sp.gr. 1·61), the containing vessel being held under running water so as to avoid any rise in temperature. The electrical contact with the specimen should be so arranged that no foreign metal is immersed in the solution, *e.g.* by the use of a pinch-clamp above the level of the liquid. If, however, it is desired to polish the whole surface of the specimen, a piece of tin wire must be inserted through a hole in it; this arrangement has the disadvantage that mechanical deformation may result. Any oxide layer on the specimen (due to thermal treatment) is first removed, and the temperature is adjusted to 15° to 22° C. and the current-density to 9 to 15 amp. per sq.dm. (calculated on the total area of the anode); these conditions should be maintained during electrolysis, the latter by adjustment of the resistance, and they correspond with the dissolution of about 3 mg. of tin per sq.cm. or a layer 0·005 mm. thick per minute. When the polishing is complete (as indicated by the bright appearance of the specimen) the anode is removed quickly (without breaking the circuit), washed rapidly in succession under running tap-water (for 2 minutes), distilled water, alcohol and ether, and finally dried in a stream of compressed air which has been filtered through cotton-wool. If the specimen is complicated in shape, two cathodes, each placed 4 cm. away from it, are desirable. So long as the solution is stored in a glass-stoppered bottle and away from access of moisture, it may be used continuously until it is no longer possible to obtain a brightly polished surface; the deepening of the colour of the solution does not impair it. Etching or corrosion figures, the shape and orientation of which are characteristic of the crystals present, may be obtained by the use of a solution prepared from 130 ml. of glacial acetic acid and 50 ml. of the perchloric acid. The voltage should then be 15 volts D.C., and the current-density 3 to 6 amp. per sq.dm. of total anode area when the specimen is immersed in the liquid, as well as subsequently; this is ensured by making the necessary adjustments during a preliminary experiment on a similar specimen of tin; if necessary, an insulating varnish may be applied to the portions of the specimen which it is not desired to treat. Cooling and

stirring are unnecessary, and the duration of electrolysis varies from 100 to 200 seconds according to the effects desired. The polished and well-dried specimen should be immersed with the current already switched on. Microscopical examination after electrolytic polishing reveals only the boundaries of the individual crystals. The etching process, however, enables small crystals (which are etched more rapidly), twins and other peculiarities to be seen. Various applications of the method are described and illustrated by 22 photomicrographs (magnifications up to $\times 1850$). It is demonstrated that Chempur tin (99·99 per cent. Sn) when cast and unannealed, has a heterogeneous structure which persists after rolling-down from 1·5 to 0·36 cm. ("rolling-ratio," 76 per cent.), and disappears only after prolonged annealing (*e.g.* for 384 hours at 200° C.). Since tin of 99·9998 per cent. purity does not show these heterogeneities, these are ascribed to the presence of metallic impurities; the impure metals probably crystallise separately after the formation of a "skeleton" of pure tin. The changes in structure that occur in tin (99·99 per cent.) and tin-antimony alloys (0·5 to 3 per cent. Sb) when rubbed with an abrasive (*e.g.* fine emery paper, grade 06) have also been studied, the specimens having previously been rendered homogeneous, by prolonged thermal treatment at 200° C. It is shown that under these conditions the layer of tin so deformed is 0·20 to 0·25 mm. thick, and that it has acquired a new structure consisting partly of small crystals formed by recrystallisation at or slightly above room temperature, and partly of needle-shaped structures ("cold-working figures"). With the alloys a zone of anomalous structure is also produced, but as a rule only cold-working effects (orientated in the direction of abrasion) are observed without the recrystallisation effects. Annealing for 15 minutes at 200° C. is sufficient to remove these figures, and to re-form the normal type of crystals. It is claimed that such effects could not be investigated by the usual methods of polishing and etching, as these would in themselves induce changes in the structure of the specimen. J. G.

Reviews

TEXTBOOK OF ORGANIC CHEMISTRY. By GEORGE HOLMES RICHTER. Pp. viii + 711. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1938. Price 20s. net.

In the preface it is stated that this textbook is directed specifically to that large body of ambitious students who wish to study organic chemistry and gain more than a superficial knowledge of the subject. With this in view the material is arranged so that emphasis falls on the fundamental principles of the science, namely, nomenclature, synthesis, reactions and properties. The aim of the book is to help the first-year student of organic chemistry to understand the subject. The work does not pretend to be an advanced exposition and does not therefore over-emphasise the importance of the electron theory of valency, as a comprehensive study of this subject would involve an intimate knowledge of higher mathematics and physics.

The book is divided into thirty-two chapters, the first twenty-one (456 pages) being devoted to the aliphatic series. Then follow nine chapters (191 pages)

dealing with the aromatic series. The last two chapters (33 pages) give a brief description of the heterocyclic series, including the more important members of the alkaloid group. Each chapter concludes with a set of problems based on the work dealt with. The index occupies thirty-one pages.

The author has done his work well and has given to the student a valuable and concise treatise on the subject, which should be of use to him long after he has completed his first year. The book is well produced and is practically free from errors and misprints. The numerous diagrams and tables are represented very clearly. The reviewer has no hesitation in recommending the work to all students and teachers of organic chemistry. R. H. SLATER

LEHRBUCH DER ORGANISCH-CHEMISCHEN METHODIK, I BAND: ANALYSE UND KONSTITUTIONS-ERMITTLUNG ORGANISCHER VERBINDUNGEN. 6 ED. Dr. HANS MEYER. Vienna: Julius Springer. Price, stitched RM.57; bound RM.59.70.

This volume of 886 pages contains an extraordinarily large amount of information. The descriptions of what may be called the laboratory arts of organic chemistry—the choice of solvents for crystallisations, the drying of solvents and other practical details of manipulation—are of great utility and are given in more detail than in any other book with which the reviewer is acquainted.

Following a detailed account (pp. 115–208) of the quantitative analysis of organic compounds containing the usual elements, there is a very useful collection (pp. 210–268) of the various methods available for the estimation of other elements, from aluminium to zirconium, which may occur in organic compounds.

The main portion of the work (pp. 361–816) gives a detailed and well documented account of the methods for the characterisation of the more important groups and radicals and for their estimation: new methods are well represented and the references to quite recent literature are very numerous.

A chapter of twelve pages gives an account of the recently developed methods of separation by chromatographic adsorption.

It is unfortunate that the cost of this useful work should be so high.

J. KENYON

THE STRUCTURE AND COMPOSITION OF FOODS. Vol. IV. By ANDREW L. WINTON and KATE BARBER WINTON. Pp. xxxiii + 580. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1939. Price 45s. net.

This volume, the fourth of the series, opens with addenda to Volumes II and III, the greater portion of which is concerned with vitamins. It is an interesting commentary on the times that every book published on the science of food must now detail the latest chemical data dealing with the structure of the vitamin molecules. Why, the reviewer fails to see. The latest theories regarding the molecular structure of many of the major constituents of the foods mentioned are not recorded. One can only assume that the chemistry of vitamins is of scientific "news value"! The addenda are interesting but not important.

The body of the book is divided into four parts.

Part I. Saccharine Products.—The sections dealing with cane and beet

sugars, maple sugar, honey, glucose and caramel give a good resumé of the preparation and chemistry of these products, including a large number of references, many useful, but some out of date. Nevertheless, there is little information given concerning the various grades of white and brown sugars encountered in commerce in England.

The inclusion of sections on sugars obtained from less common sources (palms, for example) is unusual and useful.

Part II. Alkaloidal Products.—In this section there is much information gathered together concerning products other than the main products, tea, coffee and cocoa. The clearest passages are those concerned with the macro- and micro-structure of the different plant products, a subject upon which the authors are recognised authorities. The lack of critical selection of data—noticeable throughout the book—is very obvious here, for many methods of analysis and results of analyses varying in age from 20 to 40 years are quoted. References to literature, however, are quite representative of modern thought. One might regret the lack of references to the work of Knapp on cacao fermentation. The reviewer gathers that the authors are not really conversant with the subject on which they write, otherwise such phrases as the following would not appear. "The seeds" of *Theobroma cacao*, "freed from the adhering pulp, are sun-dried either immediately or after being subjected to a fermentation, continuing for several days, that improves the flavor" (p. 114). "Some manufacturers also remove the minute radicles known in the trade as 'germs.' " (p. 114). "The gain in fiber" on roasting " . . . is doubtless due to the formation of charcoal . . ." (p. 120). They also quote seriously a formula attributed to Elsson for a substitute for cacao butter, consisting of a hydrogenated fat, lanoline and yellow wax (p. 131).

Part III. Spices and Extracts.—The characteristics chiefly praised in the review of the first volume of the series are perhaps best demonstrated in this part, for the macroscopic and microscopic descriptions are clearly stated and the drawings well chosen. The authors naturally deal with the subject-matter from the American aspect. Presumably, for example, the adulteration of dried mint by ailanthus leaves is never practised in the States, since this possibility is not mentioned in the text. Nevertheless, this is by far the best section of the book.

Part IV. Leaven.—The idea of this may be gathered from the following:—"Yeast may be defined as *biological* leaven and sodium bicarbonate and baking powder containing it as *chemical leaven*" (p. 465).

As with the vitamins, this section appears to be out of balance with the specialised treatment of the enzyme and co-enzyme systems in yeast. Certain phrases appear to suggest an incomplete appreciation of the implications, as, for example, "albumin changes little up to liquefaction, the increase in soluble nitrogen being gradual" (p. 469). "Neuberg . . . , by adjusting the volume of the medium to the dry weight of dried yeast, was able to stop the fermentation of hexose diphosphate at the pyruvic acid stage" (p. 488). The reviewer does not understand why the formula of lactic acid is expressed in the form $C_3H_6O_3 + C_3H_6O_3$ (p. 489). The following quotation is somewhat amusing. "As compared with yeast, it (baking powder) is a time saver, and time in the Western World is money" (p. 524). Another curious statement is that practically all brands of baking powder

contain "starch or less often flour, added to improve the keeping power and to standardise the carbon dioxide liberated, which incidentally adds somewhat to the nutritive value" (p. 525). Notwithstanding these criticisms, this part, shorn of certain irresponsible comments and ambiguities, represents a useful contribution to food literature.

In conclusion, the reviewer would class this volume between the good first two volumes and the more unsatisfactory third volume. L. H. LAMPITT

PHYSICAL CONSTANTS OF HYDROCARBONS. Vol. I. By GUSTAV EGLOFF. Pp. 403. New York: Reinhold Publishing Corporation; London: Chapman & Hall. 1939. Price 45s.

This volume, the first of a projected series of four, has as its sub-title "Paraffins, Olefines, Acetylenes and Other Aliphatic Hydrocarbons." Subsequently, the cyclic and aromatic series will be dealt with, and the final volume will review the relationships suggested by the data thus collected, between members of each homologous series, and between one homologous series and another.

The present volume aims at recording physical constants for every open-chain hydrocarbon isolated and identified, whether from vegetable, animal or mineral sources, up to November, 1938. Standard tables and reference-books have been laid under contribution; but, owing to the great and increasing activity in this field within the last few years, the major part of the material has been collected from individual papers and from private communications, and has not previously been brought together. It is not easy to estimate the degree of completeness with which the task has been accomplished. Random sampling suggests that any omissions which may occur must be very few and trifling; for example, although Whitmore and Southgate's publication on tetramethylpentane (*J. Amer. Chem. Soc.*, November, 1938) has apparently not been read, a private communication from the same authors is quoted, giving all the published information except the refractive index.

The constants generally given are melting-point, boiling-point, specific gravity and refractive index. When a number of determinations of a single constant are available, a preferred figure has been evaluated from these, each being weighted in accordance with its probable error where stated, and otherwise in accordance with the author's estimate of its accuracy, based on the technique described in the original paper for the preparation and examination of the compound concerned. Readings for a variety of temperatures and pressures are given, if available; and, where the range of values justifies it, coefficients are deduced for calculating constants at other temperatures and pressures as required. The Geneva nomenclature, applied in a methodical way which is carefully explained and which avoids the need of an index, is used throughout, and supplemented by a graphic representation of each carbon-skeleton involved. When the only published data refer to mixtures of isomers (*e.g.* many cases of geometrical isomerism in the olefine series, or paraffins of high molecular weight where the number of isomers may approach millions) the fact is stated.

The work, when completed, cannot fail to be of great value in both pure and applied chemistry. To the pure chemist it opens much ground, not hitherto

surveyed, on which to base correlations between properties and molecular structure. To many industrial chemists it will be an invaluable work of reference. The advances of recent years in fuel and lubricating oil technology have, to a considerable extent, revealed the dependence of the industrial usefulness of different oils upon the presence and proportion of various specific components; while the synthesis of alcohols, ketones and other solvents from cracked hydrocarbons has also been greatly developed. The identification of individual hydrocarbons must thus be of increasing importance to workers in these fields. The survey promised in the fourth volume should be welcomed by chemists, as providing both a basis for inductions of theoretical significance and an instrument enabling the technologist to forecast properties which would make it desirable to isolate or synthesise hydrocarbons that might otherwise have escaped attention. E. G. KELLETT

PLANT BIOLOGY. By H. GODWIN, M.A., Ph.D. Pp. x + 308. 3rd Edition. Cambridge: The University Press. 1938. Price 7s. 6d.

Since the previous edition was published six years ago, *Plant Biology* has been revised and enlarged, and there is little doubt that the book has been considerably improved by the additions made. The most important of these are the entirely new chapters on "The Fern Plant" and "The Flower and the Seed," so that the book now tells the complete story of the biology of plant life from the one-celled organism at one end of the evolutionary scale to the flowering-plant at the other. The story is told with the medical student, for whose especial benefit it is primarily intended, very definitely in mind, and as the title implies, the book is much more a text-book of biology than of botany. Moreover, the examples that the author takes to illustrate his theme are chosen with a view to the usefulness, in the student's later work, of the knowledge thus gained; thus more than the usual amount of space is devoted to a discussion of the biology of bacteria and fungi.

The book should prove of great value to those whose work lies on the borderland of chemistry and the biological sciences, and it can also be warmly recommended to others who may be less immediately concerned with plants, as a very clear and interesting account of how plants function. F. A. ROBINSON

THE PRINCIPLES OF ELECTROCHEMISTRY. By DUNCAN A. MACINNES. Pp. 478. New York: Reinhold Publishing Corporation; London: Chapman & Hall. 1939. Price 30s. net.

In this volume the author has endeavoured to present a unified treatment of the principles of electrochemistry on the basis of the concept of free energy as expounded by Gibbs. He traces the relationship between chemical potential and the activity of ions and then proceeds to discuss the Debye–Hückel Theory of Strong Electrolytes in regard to the assistance which it gives in the calculation of mean ionic activities from the E.M.F.'s of appropriate cells. No serious difficulties are encountered until "cells with liquid junctions" are considered, when the author is careful to point out, though in the opinion of the reviewer not with sufficient emphasis, that in order to apply thermodynamic equations to these

cells, it is necessary to know the *ionic activity of a single ion*, for the determination of which no method has yet been devised. In consequence, non-thermodynamic assumptions are introduced. The author is careful to indicate when resort is made to these assumptions. This constitutes a very serious and, at the present, an insurmountable difficulty in the application of the Activity Theory to solutions of electrolytes when being investigated by means of this type of cell. Incidentally, cells of this type find the most practical application in the analytical laboratory. The difficulty is encountered in the electrometric methods of determining pH and it is also involved in the computation of the potentials of standard half-elements, *e.g.* the normal calomel electrode.

The treatment accorded in the book to pH determination, oxidation-reduction potentials and potentiometric titrations has a distinct bias towards the mathematical theory. From the standpoint of the experimentalist, the chapters on these subjects are too inadequate to be of much service.

A comparatively small part of the book is devoted to the Debye–Hückel–Onsager theory and its use in the interpretation of the electrical conductivity of aqueous and non-aqueous solutions. A very brief survey of conductometric titrations follows.

The remaining chapters are of a rather miscellaneous character. They deal with subjects such as Structure of Organic Acids and Bases in relation to their Ionisation Constants, Dielectric Constants, Electrokinetic Phenomena, Passivity and Overvoltage. The treatment of these subjects is incomplete, cursory and disjointed. It is surprising that the general principles underlying electrolytic preparations and depositions find no place, whereas overvoltage and passivity are especially singled out for discussion. In connection with overvoltage there is not even a reference to Glasstone's work.

It should have been mentioned that in the preliminary chapters, Dr. MacInnes discusses the Classical Theory of Arrhenius and also provides a lengthy chapter on Electrical Transference. It is with his investigations on this subject that Dr. MacInnes' name is largely associated.

The theoretical development of the subject-matter throughout the main part of the volume certainly makes the treatise of considerable importance to advanced students and to post-graduates. It is hardly likely to be of much help to the elementary student, for in order to follow the subject intelligently as discussed in the book, it is essential that the student should have previously obtained a sound grasp of the more advanced principles of thermodynamics. The author's aim appears to have been to write a connected mathematical exposition of electrochemistry from the standpoint of thermodynamics and modern theory, and in this respect he has undoubtedly succeeded. It is felt, however, that this method of attack has been rendered possible by the omission of much that would be valuable from the practical point of view.

The book is exceedingly well produced, but its price is perhaps a little high.

H. T. S. BRITTON

ANNUAL REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY. 1938—Vol. XXIII. Issued by the Society of Chemical Industry. Pp. 856. Price to members, 7s. 6d.; to non-members, 12s. 6d.

The present volume has forty pages more than the last, and two new chapters appear, their titles being "Plastics" and "Explosives." Some of the twenty-six chapters have slightly modified titles, but their main subject-matter is the same as last year. The writing of each chapter and the reading of the very great number of papers to which reference is made, is a laborious task, and it is therefore not surprising to find the names of a number of new contributors in the table of contents.

Though most of us are specialists in one or two branches of our science with which the present work deals, few among us do not find it profitable to scan the progress made in other branches; such a book as this makes it much easier and more agreeable than the actual searching among hundreds of recent papers. Indeed, as almost everyone must know, one of the main purposes of these Reports is to assist the reader easily to make a choice of the papers most likely to interest him.

The literary style and all the other attributes of the volume under review are well up to the excellent standard of its predecessors. As an indication of the extent of the ground covered it may be mentioned that the name-index has some 4500 entries and the subject-index about 2900, the former being approximately the same as last year, the latter about 200 more. A count of the number of original papers referred to in the text gave a figure of over 4000. A careful search for errors of expression, punctuation and printing, revealed none of consequence and very few in actual number.

It is fascinating to follow in certain sections how the main interest shifts from year to year; in the section dealing with fine chemicals and medicinal substances, to give only one instance, formerly it was anti-malarials, then hypnotics—the veronal derivatives and so forth—and to-day it is drugs of the sulphanilamide class and chemotherapy in general.

The total benefit to mankind represented by the progress made in chemistry even in one year must be very great; in this benefit even animals and plants share—as witness the advances made in our knowledge of parasiticides and fungicides, their uses and mode of application. Of all this progress adequate notice is taken here.

At the modest price charged for the book it is safe to say that its issue represents more a labour of love than a source of profit. To those whose work is in the field of applied chemistry it will prove indispensable, and even to the "pure" chemist it may be recommended as being more than likely to provide fruitful ideas. This is borne out by the fact that some of the research in applied chemistry receives mention in the companion volume on pure chemistry issued by the Chemical Society.

F. W. EDWARDS