THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

NORTH OF ENGLAND SECTION

THE Seventh Summer Meeting was held at the Savoy Hotel, Blackpool, from June 26th to 29th. The attendance was fifty-six, including many ladies.

The Chairman (Mr. Arnold R. Tankard, F.I.C.) presided, and among those present were the following:—Past Presidents (Mr. J. Evans and Mr. F. W. F. Arnaud, with Mrs. Arnaud); Editor of THE ANALYST (Dr. C. A. Mitchell); Mr. E. M. Hawkins, Miss Bradford and Miss Elliott.

The Chairman extended a cordial welcome to all members, particularly those from the south and those attending for the first time.

On Saturday morning, Mr. A. L. Bacharach, M.A., F.I.C., read a paper, illustrated by lantern slides, entitled "The Abolition of Vitamins." A vote of thanks to Mr. Bacharach was proposed by Prof. T. P. Hilditch and seconded by Miss Roberts.

A resolution was unanimously passed expressing the greetings of the Section and affirming its loyal support to the Council of the parent Society.

Telegrams were sent to Dr. J. T. Dunn, who was absent through illness, expressing the hope for a speedy recovery, and to Prof. W. H. Roberts, regretting the inability of himself and Mrs. Roberts to be present.

The Chairman proposed a vote of thanks to the Honorary Secretary (Mr. J. R. Stubbs) for arranging the meeting. In his reply the Secretary acknowledged with thanks the help he had received from Mr. S. E. Melling. Thanks are also due to Mr. W. G. Carey, Mr. T. W. Lovett, Mr. F. J. Smith and Mr. R. W. Sutton, who, during the meeting, willingly undertook various duties.

On Sunday afternoon the party proceeded by motor through rural scenery to Newton, where tea was taken, and returned to Blackpool through the Trough of Bowland.

Each lady was the recipient of a presentation box of chocolates from Mrs. Tankard.

The Crude Protein Fraction of Fish Meal and other Meat Meals

By W. L. DAVIES, Ph.D., D.Sc., F.I.C.

In reporting the proximate analyses of meat (fish, meat and bone, whale-meat, blood) meals, it is customary to assume that no carbohydrate is present and, therefore, the fraction known as the *nitrogen-free extract* is not included. Each constituent is determined individually, and it has often been reported[1,2] that difficulty is met with in making the percentages of the constituents, determined by the usual methods, add up to 100. It has generally been assumed that the discrepancy arises from the untrue value given for the "crude protein" by multiplying the percentage of total nitrogen by the factor, 6·25; the percentage of nitrogen in the nitrogenous compounds may be less than 16 and also not a constant value from meal to meal.

The position with regard to the crude protein fraction may be summarised as follows: (*a*) the composition of the *true protein* in meat meals is variable, owing to the different amounts of the various animal proteins and bone which make up the product; (*b*) the amount of non-protein nitrogenous compounds varies from meal to meal (from 8 to 45 per cent. of the crude protein); (*c*) although the percentage of nitrogen in the non-protein nitrogenous compounds is generally lower than that of the true protein (and considerably lower than 16), the composition of these compounds is so variable that it is unwise to allot a standard factor for computing the "crude protein" equivalent of this fraction. With blood-meal, on the other hand, in which the protein composition is more uniform and the amount of non-protein nitrogen very low, the discrepancy obtained on adding up the percentages of the various fractions is small (see the first five lines of Table I).

With a view to substantiating these statements quantitatively, various commercial samples of meat and bone, fish, whale-meat and blood meals were investigated. The proximate analyses of these meals (described in Table I) were first carried out. Great care was taken in the determination of the ash. Low red heat was used in the ashing process, to prevent loss of alkali chlorides. The calcium carbonate which had been changed to the oxide during ignition was reconverted into the carbonate by moistening with ammonium carbonate solution before the final gentle ignition. The "ether extract" was determined by means of petroleum spirit, as in the method advocated by the Fertilisers and Feeding Stuffs Act.

Separation of the Crude Protein Fractions.—Two-gram portions of the meals in quadruplicate were extracted with ether in a Soxhlet extractor for 24 hours; the dry fat-free meals were treated with 40 ml. of 10 per cent. trichloroacetic acid at 70° C. for 30 minutes and allowed to stand at room temperatures for 4 hours, with occasional stirring. The acid in this process dissolved all the acid-soluble ash and precipitated the true protein. The acid-insoluble ash (see Table I) contained small traces only of calcium and phosphorus, and was mostly sand. The protein precipitate was collected on tared filter-paper (moisture-free when weighed)

and thoroughly washed with water. The filter and contents were dried at 100° C. to constant weight. Two of the quadruplicate samples were ashed to determine the amount of inorganic material in the acid-insoluble material. The other two samples were used for the determination of the trichloroacetic acid radicle combined with the protein. This was carried out by treatment of the protein, beaten up with the paper, with 40 ml. of 0·2 *N* sodium hydroxide solution at 70° C. for 4 hours. This treatment decomposed the trichloroacetic acid into chloroform and carbon dioxide, which was absorbed by the alkali. The mixture was acidified with excess of sulphuric acid, and the carbon dioxide was aspirated from the boiling solution and absorbed in 20 ml. of 0·1 *N* sodium hydroxide solution. The carbonate was determined by the usual double titration method. Corrections were made for blank determinations carried out on the original caustic soda and the alkali used for absorption. The weight of trichloroacetic acid combined with the protein was taken as the whole molecule, since the combination was assumed to occur according to the following equation:

$$X.NH_2 + CCl_3.COOH = X.NH_3.OOC.CCl_3.$$

The percentage of ash-free and trichloroacetic acid-free true protein in the original air-dry meal was then calculated. The nitrogen-content of the filter paper pulp and digested meal in the flask was then determined by the Kjeldahl method. The non-protein nitrogen in the filtrate from the trichloroacetic acid precipitation was also determined. This value, subtracted from the total nitrogen determined in the initial proximate analysis, served as a check for the true protein nitrogen. It was found that there had been only a trace of nitrogen lost as ammonia by the alkaline treatment of the true protein fraction for 4 hours at 70° C.; this shows the great stability of the amide nitrogen to mild alkaline treatment.

The percentage of the non-protein nitrogenous compounds (N.P.N.) was calculated by difference; N.P.N. compounds = meal − (moisture + ash + ethereal extract + true protein). The nitrogen-content of this fraction being known, the percentage of nitrogen was calculated.

The results of the determinations described above are given in Table I.

Discussion of Results.—The non-protein nitrogenous compounds are highest in fish meals (25 to 33 per cent. of the air-dry meal) and much lower in meat and bone meals (17·0 to 18·5 per cent.). Those in blood meals account for less than 2 per cent. of the product. The ratio of true protein to non-protein nitrogen is consequently lowest (average 1·6) for fish meals; this ratio is about 2·5 for whale-meat meals and 2·0 for meat and bone meals. With the fish meals a rough correlation exists between the amount of non-protein nitrogenous compounds and the percentage of nitrogen in that fraction; the higher the percentage of non-protein nitrogenous compounds, the lower the percentage of nitrogen in the fraction. The number of the values for the other types of meals does not justify any conclusions being drawn.

In every instance, except in samples B and K and the blood meals, the percentage of nitrogen in the non-protein nitrogen fraction was less than 16·0, and in 7 out of 12 meat meals the percentage of nitrogen in the same fraction was less than that in the true protein. Only one sample (fish meal) showed a higher percentage of

nitrogen than 16 per cent. in the true protein. The true proteins of the blood meals contained less than 16 per cent. of nitrogen, but the non-protein nitrogen fraction counterbalanced this deficiency by having a very high nitrogen-content.

TABLE I

NITROGEN DISTRIBUTION IN MEAT MEALS

Percentages on Air-dry Meals

Material	Meat and bone meals			Fish meals					Whale-meat meals			Blood meals	
	A	B	C	D	E	F	G	H	I	K	L	M	N
Moisture ..	11·93	10·23	5·79	15·75	14·06	11·10	10·80	10·22	10·52	5·19	11·56	12·59	9·74
Pet. spt. extract	5·88	4·56	5·77	2·60	5·22	3·25	3·68	4·90	5·89	10·43	5·48	0·25	0·46
Ash ..	27·75	25·25	28·06	22·27	19·46	22·28	16·49	21·94	17·36	19·56	21·50	4·33	4·42
Crude protein N × 6·25 ..	49·08	52·69	47·19	55·81	58·56	59·06	59·44	57·88	58·56	59·81	54·81	81·19	84·58
Total	94·64	92·73	86·81	96·43	97·30	95·69	90·41	94·94	92·33	94·09	93·35	98·36	99·20
Crude protein (calculated by diff.) ..	54·44	59·96	60·38	59·38	61·26	63·37	69·03	62·94	66·23	64·82	61·46	82·83	85·38
True protein ..	35·99	42·72	41·19	33·11	34·14	38·42	36·07	37·84	44·60	47·45	50·55	81·00	84·00
Non-protein N compounds	18·45	17·24	18·19	26·27	27·12	24·95	32·96	25·10	21·63	17·37	10·91	1·83	1·38
True protein nitrogen ..	5·14	5·57	5·39	5·03	5·43	6·23	5·71	6·06	6·47	6·71	7·13	12·55	13·26
Non-protein nitrogen ..	2·71	2·86	2·16	3·90	3·94	3·22	3·80	3·16	2·90	2·86	1·64	0·44	0·27
Acid-insoluble ash	2·49	1·83	4·16	0·48	0·54	1·28	0·48	0·89	0·55	0·35	0·26	0·05	0·08
True protein N / Non-protein N	1·90	1·95	2·50	1·29	1·38	1·94	1·50	1·92	2·23	2·35	4·35	28·50	49·10
N in true protein, per cent. ..	14·29	13·05	13·08	15·19	15·91	16·21	15·83	16·02	14·51	14·14	14·10	15·49	15·79
N in non-protein N compounds, per cent. ..	14·69	16·59	11·88	14·85	14·53	12·90	11·53	12·59	13·41	16·47	15·03	24·04	19·56
Factor for crude protein ..	6·93	7·11	8·00	6·65	6·54	6·71	7·26	6·43	7·07	6·77	7·01	6·38	6·31

The factor by which the total nitrogen has to be multiplied in order to give the true crude protein value is, for blood meals, very close to the usual factor, 6·25; for the other meals the factor, however, varies from 6·54 to 8·00. Whale-meat meals, as a group, show the least variation in the factor, namely, 6·95 ± 0·09; the value of the factor for fish meals is 6·72 ± 0·29, and, for meat meals, 7·35 ± 0·31. This makes a change in the factor out of question. An adjustment of the factor depending on the amount of non-protein nitrogen is also not permissible, owing to the great variations in the composition of this fraction. Table I brings out clearly the considerable variation met with in the composition of meat by-products.

There are very small amounts of woody material in some samples of fish meal and only very small traces, if any, in the other meat meals. The amount, however, is too small to invalidate the general findings given in this paper. In any case, since it is not the custom to report fibre and nitrogen-free extract in meat meals,

the small trace of wood would be included in the protein. The same can be said of the glucose (from glycogen), which is present in small amounts.

SUMMARY.—The "crude protein" of various meat meals has been separated into true-protein and non-protein nitrogen fractions. The variation of the percentage of nitrogen in these two fractions has been discussed. Generally, the nitrogen-content of the non-protein nitrogen fraction is lower than that of the true protein. Meat by-products, however, are of such a variable composition that it is futile either to suggest the use of a new factor by which the percentage of total nitrogen has to be multiplied in order to get the true crude protein value or to adjust the factor in relation to the non-protein nitrogen content.

REFERENCES

1. J. G. Sherratt, ANALYST, 1935, **60**, 170
2. G. A. Lawrence, *ibid.*, 1935, **60**, 611.

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UNIVERSITY OF READING

The Estimation of Carotene in Agricultural Products

BY W. S. FERGUSON, A.I.C., AND G. BISHOP, M.Sc., A.I.C.

IN recent years carotene has become the subject of much research work because of its vitamin *A* activity, and its determination is now being undertaken by a number of agricultural chemists who realise its importance in the nutrition of farm stock.

Although in the intensive rearing of pigs and chickens additional vitamin *A* is usually given in a cod-liver oil supplement, farm stock have to depend almost entirely on the carotene in their foodstuff as a source of vitamin *A*. It is of great importance, particularly during the winter months, to ensure that the animals receive a sufficiency of carotene, not only for their own well-being, but also for the effect on the health of the population.

It is well known that cows fed on a diet containing a plentiful supply of carotene yield milk and butter rich in vitamin *A* and carotene; as these foodstuffs figure so largely in the dietary of the people, it is desirable to maintain in them a high level of vitamin *A* potency. The liver and other organs of such animals are also particularly rich in vitamin *A* potency, and these too contribute to the requirements of the consumers.

Methods for the estimation of carotene in agricultural products have been worked out at this Station; the method given for grasses and fodders should find general application for other types of material, such as liver, with minor modifications.

THE ESTIMATION OF CAROTENE IN GRASSES AND FODDERS.—The material is finely chopped, and the required amount is weighed out into a 300-ml. flat-bottomed

flask. The quantity used depends on the amount of carotene and moisture in the material. With hay, fresh grass and other fresh green crops, 10 g. are taken, whilst with dried material moderately rich in carotene, such as artificially dried grass, 2 g. are sufficient. Material is weighed out at the same time for the determination of the moisture-content, as it is advisable to report the carotene-content on a dry-matter basis, owing to the fluctuations in the moisture-contents of green crops.

Fifty ml. of 20 per cent. aqueous potassium hydroxide solution are placed in the 300-ml. flask, and the mixture is boiled gently for two hours under reflux. When dried material is being examined, boiling for one hour is sufficient. The flask is cooled, and the contents are filtered under reduced pressure through a Hirsch funnel previously packed with moistened cotton-wool. The residue is washed three or four times with ether-saturated water, the last two washings being carried out by transferring the residue to a beaker, stirring it vigorously with the ether-saturated water and filtering. When the washings are colour-free, the residue is again transferred to the beaker and extracted similarly with small quantities of pure acetone until they also are colour-free; about 30 to 40 ml. are usually necessary for this extraction. A considerable quantity of yellow pigment is extracted by the acetone; this consists of a mixture of carotene and xanthophyll.

The total aqueous and acetone extracts are transferred to a 500-ml. separating funnel and extracted exhaustively with ether, 3 or 4 extractions usually being sufficient. The combined ethereal extracts (amounting to 150 to 200 ml.) are then washed 4 times with water.

The ethereal solution is transferred to a measuring cylinder and its volume noted. The solution is then ready for colour matching, but, as the colour is usually too dense to match directly, appropriate dilutions are made. A Lovibond tintometer (B.D.H. Pattern) is used for the matching, and two or three comparisons are made between the range of 2 and 5 yellow units. To obtain perfect colour matching it is necessary to use 0·2 or 0·3 red units in conjunction with the yellow units.

The total carotenoids, expressed as carotene, are calculated from the curve prepared in this laboratory (Ferguson[1]).

The total carotenoids consist of carotene and xanthophyll, the relative proportions of which are fairly constant in fresh grasses—1 part of carotene to 2·1 parts of xanthophyll. The estimation of the relative amounts of these constituents is carried out by the methyl alcohol and petroleum spirit partition method.

Partition of Carotene and Xanthophyll.—One hundred ml. of the total carotenoid solution are transferred to a 250-ml. distilling flask, and the ether is removed in a stream of nitrogen on a water-bath having a temperature not above 40° C. The residue is washed out of the flask into a 200-ml. separating funnel with about 100 ml. of petroleum spirit. If the material does not dissolve readily, a few ml. of ether can be used, followed by 92 per cent. methyl alcohol. The petroleum spirit solution is extracted exhaustively with colour-free 92 per cent. methyl alcohol, which removes the xanthophyll.

If the petroleum spirit fraction is cloudy, it is clarified by adding a few drops of ethyl alcohol, and the carotene-content is then determined by matching it in a

Lovibond tintometer, after the necessary dilutions, as for the total carotenoids. Similarly, the xanthophyll is estimated on the methyl alcohol extract.

From the two values obtained, the ratio of carotene to xanthophyll is determined, and from this ratio the carotene-content of the original extract is calculated.

The reason for applying this ratio to the total extract, rather than calculating the carotene-content from the petroleum spirit extract, is that during the partition there is a loss, which may amount to 10 per cent. of the total carotenoids present. Usually, the loss is about 5 per cent., and falls on both of the constituents, as is shown by the following experiment.

Five partitions were carried out on a total carotenoid solution obtained from dried grass. The losses of carotene and xanthophyll are shown in Table I.

TABLE I

	Percentage losses of carotenoids during partition		
No.	Total carotenoids	Carotene	Xanthophyll
1	4·3	5·3	3·9
2	7·2	8·2	6·8
3	5·4	10·1	3·8
4	0	0	0
5	3·6	2·9	3·9
Average	4·1	5·3	3·7

The relative losses of carotene and xanthophyll are somewhat irregular, but little error will be introduced into the carotene estimation if it is assumed that the carotene and xanthophyll suffer equal losses.

Duplicate estimations of the carotene-content of fresh grass show very good agreement, usually within 1 per cent. With dried fodders larger variations have been obtained, and differences up to 5 per cent. may be expected.

Loss of carotenoids may possibly occur during the processes prior to the matching of the ethereal solution of the total carotenoids, and this possibility has not been investigated critically. Experiments have shown that no advantage is gained by carrying out this stage of the estimation in the dark or in an atmosphere of nitrogen to minimise losses due to oxidation, and this would suggest that any losses, if they occur, are negligible. Also, from the agreement obtained between duplicates, it would appear that even if such possible losses are sometimes less inconsiderable, they are systematic, and therefore would not detract from the comparative value of the results.

METHOD FOR THE ESTIMATION OF CAROTENE IN MILK AND BUTTER.—For the estimation of carotene in milk a portion of the milk-fat is separated and its colour is matched, after suitable dilution, in the Lovibond tintometer.

The separation of fat from milk by extraction with ether or petroleum spirit is complicated by the formation of emulsions very difficult to break down. For this reason the extraction, in the method described, is not quantitative. However, carotene is all in solution in the milk-fat, and it is unlikely that any preferential extraction of carotene will arise, so that the extracted fat will be representative of the total fat in the milk.

To 200 ml. of milk in a 500-ml. separating funnel are added 20 ml. of 25 per cent. aqueous potassium hydroxide solution, and the mixture is gently shaken. One hundred ml. of ethyl alcohol and 100 ml. of petroleum spirit (b.p. below 40° C.) are added separately, with careful shaking after each addition. If the shaking has been done with care, the emulsion breaks down in a short time and the petroleum spirit layer separates. The aqueous solution is run off, and the petroleum spirit extract is transferred to a round-bottomed flask by pouring the extract from the top of the funnel and allowing it to filter through cotton-wool. The petroleum spirit is distilled off in a stream of nitrogen on a water-bath with a temperature not exceeding 40° C., and the last traces of the solvent are removed under reduced pressure.

To portions of 2 ml. of the butter-fat contained in a 10-ml. measuring cylinder are added varying amounts of petroleum spirit, and the colours of the solutions are compared with the yellow glasses of the Lovibond tintometer. The equivalent of the Lovibond units in terms of total carotenoids (mg. per litre of solution) is then read off from the curve already mentioned.

The carotenoids of butter consist almost entirely of carotene, only about one-fifteenth of the colour being due to xanthophyll.

Example of Calculation of Results.—To 2 ml. of milk-fat were added 6 ml. of petroleum spirit. The solution was equivalent to 4·5 yellow Lovibond units, or 2·41 mg. carotene per litre of solution. The sp.gr. of the fat being taken as 0·9, the carotene contained in 100 g. of milk-fat was

$$\frac{2{\cdot}41}{1000} \times \frac{8}{2} \times \frac{14}{15} \times \frac{100}{0{\cdot}9} = 1{\cdot}00 \text{ mg.}$$

The results can be expressed in terms of the original milk by estimating the fat-content of the milk and making the necessary calculation.

In Butter.—The butter is clarified by filtration, and the carotene is then estimated in the butter-fat as described above.

Reference

1. W. S. Ferguson, Analyst, 1935, **60**, 680.

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Cloth Oils and Catalysts in the Mackey Test: Oxidation of Olive Oil

By W. GARNER, M.Sc.

THE suitability of an oil for the lubrication of wool before combing is generally considered to depend upon its degree of freedom from poly-ethenoid bodies, such as the linolic glycerides. Thus, olive oil is more suitable than arachis oil, and arachis oil than cottonseed oil.

The amount of oil applied to wool in the worsted industry is only about 3 per cent. of the weight of the wool, and this is barely sufficient to form an extremely thin film over the fibre surface. Ideal conditions are therefore provided for the maximum oxidation of the oil by the atmospheric oxygen; the unsaturated glycerides are considered to oxidise or to polymerise into resinous bodies which are difficult to remove during scouring with soap and alkali; the unremoved resins cause various faults in later processing.

Oleic acid glycerides are oxidised much more slowly than the di- and poly-ethenoid glycerides. It would therefore seem possible to oxidise an oil selectively, for example, by "blowing" the oil under chosen conditions. In the following experiments the oil was "blown" for six hours at a temperature of 110° C. by bubbling air through it by means of suction from a vacuum pump. (In comparative tests it is advisable to blow all the oils under examination simultaneously under identical conditions.)

In view of the small drop in iodine value to be expected from complete oxidation of, say, 5 per cent. of linolic glyceride in normal olive oil, the iodine value is not a sufficiently sensitive indication of the oxidisability. The Mackey test was therefore employed. The reliability of this test has been adversely criticised in certain quarters, but in the present experiments it was found that, provided certain precautions are observed and standard conditions maintained, results can be reproduced very exactly. (*Cf.* Garner and Leach.[1] The cotton-wool used contained 0·0007 per cent. of iron.)*

METHOD.—The following details are of importance:

Jacketed Vessel.—That supplied by the makers is used.

Thermometer.—The type supplied with the instrument reads only to 400° F., which is inadequate, as it often happens that an oil will give a reading as high as 450° F., and the temperature will then begin to fall. The bulb length is ½ in., and the distance from the bottom of the bulb to the 212° F. mark is 4 in. The thermometer is set permanently in the lid, the distance between the bottom of the

* It is obvious that unless some standard be adopted for the amount of oxidising catalyst present in the cotton wool, it will be impossible to obtain concordant results. A determination of the percentage of iron, cobalt, manganese, etc., is of little use, because some portion of the metal may be present in an inactive combination, *e.g.* as rust; further, these metals differ in activity, making any computation of the total activity difficult.

A quantity of cotton wool should be set aside as a standard, to be used only for the purpose of selecting cotton for the Mackey tests. When a fresh batch of cotton is required, tests should be carried out upon a sample of olive oil, using various lots of cotton, until one is found which is equivalent in activity to the standard. The standard adopted should not be extremely pure, for a cotton containing a comparatively large amount of active catalyst magnifies the differences between oils and detects iron sensitivity.

bulb and the underside of the lid being 2 3/16 in.; in this position the bottom of the bulb is inserted to a depth of 2 in. into the cotton wool during the test.

Cotton Wool.—This must be bought in a well-carded condition, free from "neps." It should be perfectly neutral, and the iron percentage should be determined. The fluidity may also be determined, as a highly degraded cotton causes an increased oxidation of the oil.

Cards.—The face area is 7 in. × 2½ in. The "clothing" is J. S. Fillett, supplied by Messrs. Sellers & Sons, Ltd., Cleckheaton, Yorkshire.

The influence of certain variations from the above-described conditions may be reviewed briefly.

The presence of oxycellulose causes a slight, but definite, increase in the rate of oxidation.

The presence of iron, even in minute traces, causes increased rates of oxidation. This was shown by the difference in the curves in an olive oil test, in which cotton wool containing 0·00007 per cent. of iron and another cotton wool containing 0·00003 per cent. of iron were used.

The importance of iron as an oxidising catalyst was also shown by the difference in the curves obtained with a good olive oil, with and without the addition of 0·000025 per cent. of ferric oleate.

The "cards" recommended have steel filletting, but this introduces no errors. New cards, however, should be cleaned with olive oil before being used for testing purposes, as there is a surface coating of oil-soluble iron-containing matter which must be removed. It is advisable also to clean the cards after they have been used on a "bad" oil, to avoid any possibility of error. Otherwise the cleansing with methylated spirits is quite adequate.

Some oils are more sensitive to iron than others, and as this sensitivity has an important industrial bearing, especially in the examination of woolcombing oils, it is suggested that the iron sensitivity be determined by testing the oil in the Mackey apparatus with and without the addition of 0·0001 per cent. of ferric oleate.

Sampling.—Oil samples should be well shaken up before weighing, as the suspended matter present usually has a pronounced catalytic action in aiding oxidation. Comparative tests upon the effect of additions to oils should be made upon filtered samples.

Influence of Light.—Exposure, even for a few minutes, to strong light, either during or after carding, causes an increased rate of oxidation. The previous history of exposure to light of the oil sample, *e.g.* in a glass bottle in sunlight, also has a slight effect upon the Mackey test result.

Attention to these details enables duplicate tests, using the same cotton wool, to agree to within five minutes; the importance of the iron-content of the cotton wool must again be stressed.

The rates of oxidation of three representative olive oils—(i) a pure Malaga oil, (ii) a good commercial oil, and (iii) a cheap commercial oil—in the Mackey apparatus are shown at O in Figs. 1–3. The refractive index of oil No. (ii) was slightly high. The high acetyl value of oil No. (iii) suggested that it might have been washed with alkali to reduce its acidity below 5 per cent. (Assuming that free fatty acid is produced by the hydrolysis of triglyceride, 5 per cent. of free fatty acids should

correspond with an acetyl value of 12·5, maximum.) Otherwise the analytical values were normal. The Mackey results are summarised in Fig. 4.

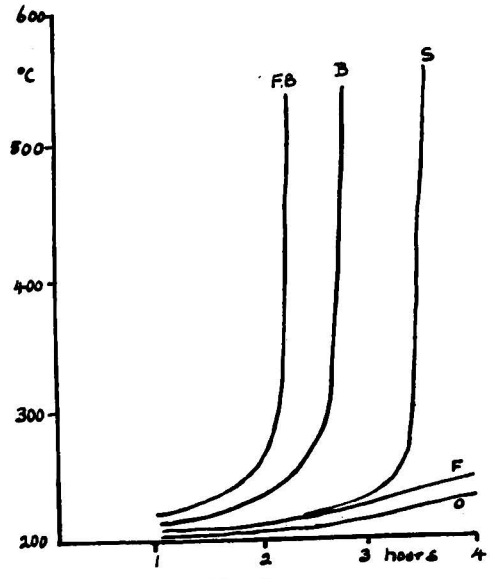

Fig. 1
Mackey tests on Oil No. (i) after various treatments

The behaviour of the oils after "blowing" for six hours at 110° C. in a glass vessel is shown at B in Figs. 1 to 3. Oil No. (ii) shows the expected improvement, but oils Nos. (i) and (iii) were, surprisingly, much more easily oxidised after blowing than before.

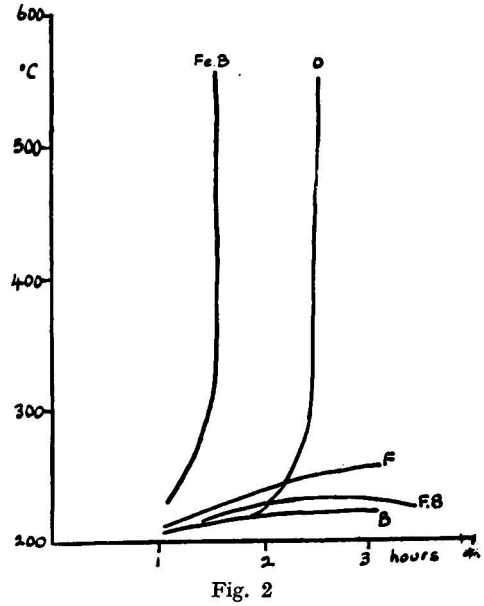

Fig. 2
Mackey tests on Oil No. (ii) after various treatments

It was thought that this behaviour of Nos. (i) and (iii) might be due to some impurity such as iron rust, which might act as a catalyst. The oils were therefore examined again after filtration through hard filter-paper. The results are shown at F in Figs. 1 to 3. It will be seen that there was a very considerable improvement in oils Nos. (ii) and (iii), whilst oil No. (i) was, of course, little affected.

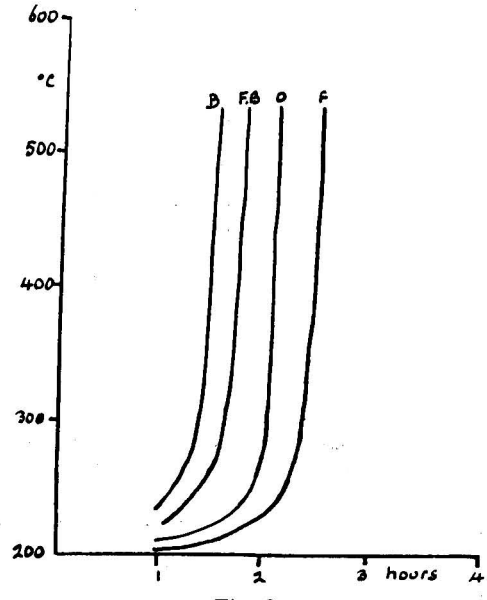

Fig. 3
Mackey tests on Oil No. (iii) after various treatments

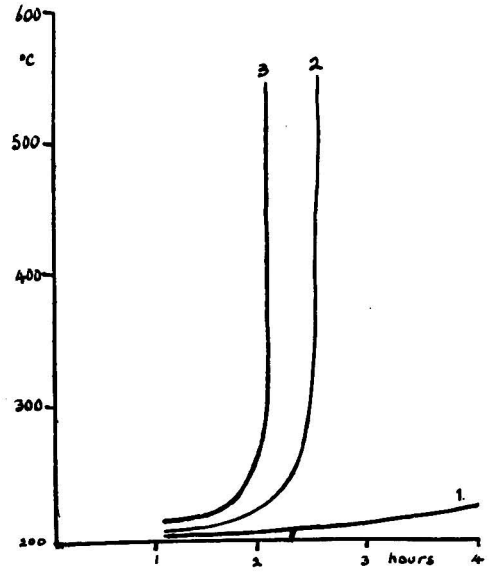

Fig. 4
Mackey tests on original oils

All the filtered oils were then blown under the standard conditions detailed, in order to effect oxidation of the linolic glycerides. The results are given at FB in Figs. 1 to 3; the filtered oils Nos. (i) and (iii) were again much more easily oxidised after blowing, whilst No. (ii) was unaltered.

TABLE I

Treatment	Oil No. (i)			Oil No. (ii)			Oil No. (iii)		
	Iodine value	M*	T†	Iodine value	M*	T†	Iodine value	M*	T†
Original	83·9	–	218	83·4	155	–	84·7	125	–
Filtered	83·8	–	220	83·4	–	259	85·1	150	–
Blown	80·0	165	–	79·6	–	226	78·3	90	–
Filtered and blown	81·9	135	–	80·0	–	230	79·3	115	–

* M = Minutes taken in Mackey test for the temperature to reach 400° F.
† T = Temperature in °F, in the Mackey test at the end of 180 minutes.

These results are summarised in Table I, from which it appears that:

(*a*) Filtration removed from oils Nos. (ii) and (iii) some body which accelerated the oxidation in the Mackey test.

(*b*) The stability of the filtered oils Nos. (i) and (ii) indicated that the unsaturated glyceride-content is not the primary cause of rapid oxidation.

(*c*) Blowing reduced the oxidisability of oil No. (ii); from (*b*) (above), it would seem that this improvement was due more probably to destruction of the substance which can be separated by filtration, than to oxidation of linolic glycerides. (Heating without blowing has practically the same effect as heating with blowing.)

(*d*) Blowing increased the oxidisability of oils Nos. (i) and (iii), indicating a development of activity in some substance (present in the original oil in an inactive form) which is not removed by filtration.

To explain these results it is necessary to postulate the existence of two catalysts (and possibly, from the behaviour of oil No. (i), an anti-oxidant also):— Catalyst A: An active catalyst, removed from the oil by filtration, and slowly destroyed by heat. Catalyst P: A potential catalyst, not removed by filtration, and slowly developed into an active form by heat.

Now let mP be the amount of activity developed by P during the time of the Mackey test, and let P be the amount of activity developed during the blowing operation.

Similarly let bA be the amount of activity of catalyst A remaining after blowing (numerically, m and b are, of course, less than unity).

Then the rise in temperature during the Mackey test is due to:

Original oil	$A + mP$
Filtered oil	mP
Blown oil	$bA + P$
Filtered and blown oil ..	P

The presence of appreciable amounts of linolic acid or its esters may, in addition, be conceded to increase the rate of oxidation of the unblown oils compared with that of the blown oils, from which linolic glycerides must to a large extent be absent. The chemical changes produced by oxidation in the Mackey apparatus are under investigation.

The behaviour of the three oils may now be explained as follows:

Oil No. (i).—This contains *P* but not *A*. Hence in the original state it is fairly stable to the Mackey test, but, after blowing, it is easily oxidised, owing to the development of the activity of *P*. The slightly increased oxidisability after filtration may be due to the removal of an anti-oxidant.

Oil No. (ii).—This oil contains *A* but not *P*. It is consequently oxidised easily in its original form, but mere filtration causes a great reduction in oxidisability, owing to removal of *A*, whilst blowing has no effect, because no *P* is present.

Oil No. (iii).—In this oil both *A* and *P* are present, the latter in large amount. Consequently, the original oil oxidises easily; its rate of oxidation is retarded by filtration (but a considerable development of the activity of *P* takes place during the time of the Mackey test); and the oxidisability is much increased by blowing which developes the full activity of *P*.

The changes taking place in blowing may be illustrated by Fig. 5 depicting the blowing of oil No. (iii). The most noteworthy point is that the increased oxidisability, as shown by the Mackey test, soon attains a constant value, indicating that this increase of oxidisability is due to the complete development of a definite amount of potential catalyst, and not to a steady progressive deterioration of the oil. The improvement shown after nine hours' blowing may be due to the pronounced polymerisation as well as to the destruction of catalyst *A*.

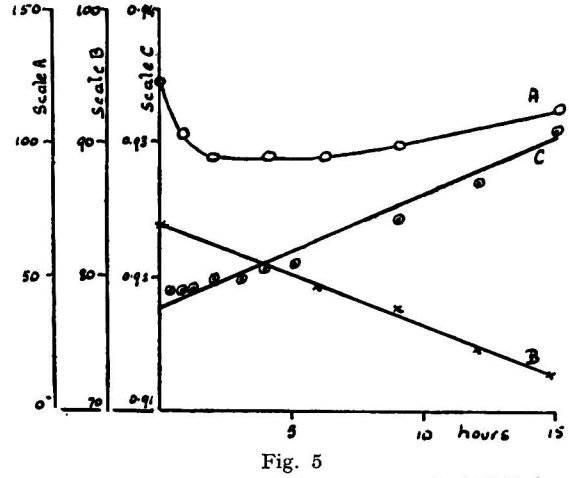

Fig. 5

Scale A: Time taken in minutes to reach 400° F. in the Mackey test by Oil No. (iii) blown for the number of hours stated. Scale B: Iodine values. Scale C: Specific gravities

The changes in certain constants are given in Table II; it should be mentioned that the acetyl value at 15 hrs. was lower than that after 8 hrs. (35·8), possibly indicating that hydroxyl groups were involved in the polymerisation.

The nature of the catalysts is still in some doubt, but the following experiments throw some light upon their constitution.

Catalyst A.—A Winchester quart of oil No. (ii) (which contains *A* mainly) was filtered with the aid of the pump. The residue on the filter-paper was mucilaginous and admixed with oil. Some of the slime was scraped off and well dispersed

TABLE II

	Oil No. (iii) Original oil	Blown for 15 hours at 110° C
Iodine value	83·9	73·1
Saponification value	196·9	199·0
Acetyl value	19·1	28·1
Acetyl saponification value ..	205·9	215·5
"Oxy acids," per cent.	1·9	5·1
Acetyl value of "oxy acids" ..	—	173·0
Free fatty acid, per cent.	4·5	5·1

in a few ml. of oil No. (i), by rubbing the mixture between glass plates. The mixture was tested in the Mackey apparatus and found to be easily oxidised (Fig. 1, curve S), showing that a catalyst was present in the slime. Blowing destroyed the catalyst.

A second lot of oil was filtered, and the slime extracted with ether. The ethereal extract after evaporation, it is interesting to note, contained about 50 per cent. of a heavy white suspension of solid glycerides. The extracted residue was dispersed in water, and a portion was added to a solution of guaiacol resin in acetone. No colour was produced, indicating the absence of "oxidases." Hydrogen peroxide was then added, upon which a faint blue colour slowly developed, this being the reaction of "peroxidases."

The remainder of the dispersion was evaporated to dryness and ignited, the residue was taken up with dilute hydrochloric acid, and the solution was neutralised and tested for metals. The total ash was 0·0006 per cent. of the weight of the oil filtered. The metals found were:—iron, 0·0003; cobalt, 0·0001 per cent.; the presence of manganese was shown by the benzidine spot-test, but no estimation was made.

The catalyst *A* may therefore be presumed to partake of the nature of a "peroxidase," but the amount present seemed rather small to explain the Mackey oxidation of oil No. (ii), and it is difficult to account for the action of a heat-labile enzymic body at temperatures above the b.p. of water. On the other hand, the metals present, if in an active form, are sufficient to account for the oxidation, but it is difficult to see how their activity could be destroyed by blowing, except by conversion to oxide. (Metals present as oxides, *e.g.* iron rust, are inactive even after blowing.) An attractive hypothesis is that heat destroys the activation by light, noted by, for example, Lea.[2]

Catalyst P.—This body is not removed by filtration. The ash of the filtered oil contained 0·0008 per cent. of iron, and gave very strong spot-tests for cobalt and manganese, together with some magnesium (from chlorophyll ?).

Its properties could be explained by assuming it to be a complex iron-containing body, such as a protein, too finely dispersed in the oil to be removed by filtration. Such a protein body would slowly be decomposed by heat, when its metal-content would form oil-soluble metallic soaps, *e.g.* ferric oleate. These would then catalyse the oxidation of the oil, acting as oxygen carriers to the unsaturated linkages. The effect of catalysts upon peroxide formation is under investigation.

As confirmation of this point of view, the following experiments may be cited: Filtered oil No. (ii) was blown in the presence of (*a*) iron rust, (*b*) metallic iron, (*c*) ferric oleate. The products from (*a*) and (*b*) showed a definite, though slight,

increase in oxidisability. The product from (*c*) was extremely easily oxidised, as shown at FeB, Fig. 2. Ferric oleate added to filtered and blown oil also gives a very oxidisable oil. Traces of manganese oleate or cobalt oleate were even more effective in increasing the oxidisability greatly. The oils containing these metallic soaps were not improved by filtration.

In a second series of experiments haemoglobin was used to simulate the action of an iron-containing protein. It was ground up with filtered oil No. (ii), and filtered to remove large haemoglobin aggregates. The oil thus produced gave an excellent Mackey test, being only very slowly oxidised, but after blowing, the oil was very easily oxidised.

It is therefore extremely probable that the potential catalyst *P* is a vegetable protein complex containing the metals, iron, cobalt, and manganese, which is very finely dispersed in the oil.

There remains the possibility that catalysts *A* and *P* have a common parent substance, or that *A* is derived from *P*, *e.g.* by storage in warm conditions. The fact that *A* can be filtered off, whilst the filtered and blown oils are not improved by filtration, does not support this possibility.

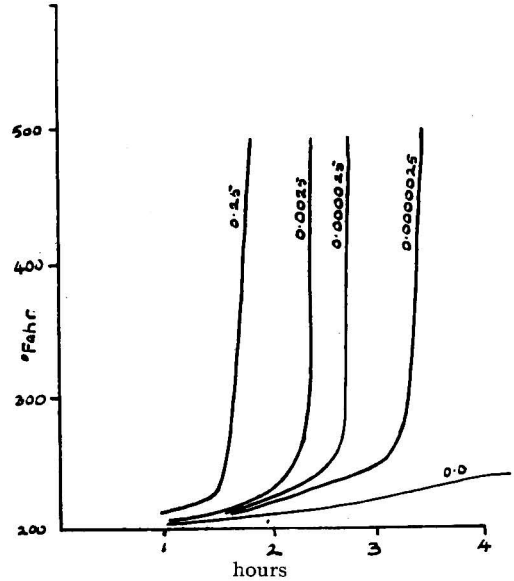

Fig. 6

Mackey tests on Oil No. (i) containing the stated percentages of ferric oleate

The remarkable influence of traces of oil-soluble ferric salts is illustrated in Fig. 6, which gives the results of adding various percentages of ferric oleate to a specific olive oil. The addition of 0·01 per cent. of ferric oleate to medicinal paraffin had no effect upon the Mackey test of the paraffin, which showed no rise in temperature.

The following sidelights upon this work may be of interest:

ANTI-OXIDANT IN OIL NO. (i).—In an unsuccessful endeavour to extract proteins by washing with various aqueous solutions it was noticed that

oil No. (i), washed with either 10 per cent. NaCl solution, 70 per cent. alcohol, or 50 per cent. alcoholic hydrochloric acid, was oxidised much more rapidly in the Mackey test, the brine wash having the most marked effect. Oils Nos. (ii) and (iii) were almost unaffected by this treatment, and it seems probable that oil No. (i) contained an anti-oxidant, natural or otherwise. This oil must therefore be supposed (despite Fig. 1) to contain a small amount of active catalyst, the action of which is inhibited by the presence of an overwhelming amount of anti-oxidant.

IRON SENSITIVITY OF OILS.—An addition of 0·0001 per cent. of ferric oleate was made to a large number of olive oil samples, and the Mackey oxidation was examined with and without such addition. It was found that some oils were much more sensitive to iron than others, probably because of the amount of natural anti-oxidant present. This is a matter of considerable importance to the textile trade, for, during the processing of wool by the worsted method, the oiled wool comes into contact, during carding, gilling and combing, with very large areas of steel pins and wires for prolonged periods. An oil which, when bought, appears to be free from oxidation in the Mackey test may, if iron-sensitive, pick up sufficient iron from the cards, gillboxes and combs to cause it to oxidise very easily when exposed to the atmosphere in a thin film on the wool; even 1 p.p.m. of ferric oleate has a pronounced effect. It is suggested that all olive oils purchased for use for wool processing be examined for their sensitivity to catalytic oxidation by adding 0·01 per cent. of ferric oleate and observing the influence of this addition upon the Mackey test.

EFFECT OF UNSATURATION.—Three portions of filtered oil No. (ii) containing 10 per cent. respectively of commercial olein, pure oleic acid (iodine value 90·1), and commercial linolic acid, with addition of 0·01 per cent. of ferric oleate to each to nullify the effect of adventitious iron in the acids, were examined in the Mackey apparatus. All three portions gave practically identical readings.

EFFECT OF SAMPLING METHOD.—The result of the Mackey test of a sample of oil drawn from the top of a barrel which had been undisturbed for some weeks, was compared with that given by the same oil after the barrel had been rolled about the floor. The greatly increased rate of oxidation shown by the latter sample was due to the presence of oxidising catalysts in the sediment in the barrel.

EXAMINATION OF OTHER OILS.—All the natural vegetable oils examined proved to contain oxidising catalysts, sometimes in considerable amounts. Treated oils often contained only potential catalysts; for example, a sample of washed, bleached and deodorised arachis oil gave an excellent Mackey test before blowing, but after heat treatment oxidised very rapidly. Synthetic esters also show the same behaviour; for example, pure diglycol oleate shows no rise in temperature in the Mackey test, but a commercial sample containing 0·2 per cent. of iron ignited after $1\frac{1}{4}$ hours. A metal-free sample of oleyl oleate was examined for eight hours in the Mackey apparatus without any rise in temperature being observed, despite its unsaturated nature. Pure ethyl oleate behaves in the same way.

SUMMARY.—1. Natural oils contain oxidising catalysts which have a very considerable influence upon their oxidisability. Two types of such catalysts, *viz.* active and potential, are described.

2. The rate of oxidation of an oil in the Mackey test is primarily determined by the content of active catalyst, and not by the degree of unsaturation of the oil. There is little relationship between the iodine value of an oil and its behaviour in the Mackey apparatus.

3. The active catalyst may be determined by carrying out a Mackey test upon an oil. The potential catalyst may be determined by heating the filtered oil for 3 hours at 110° C. and carrying out a second Mackey test.

4. The determination of the amount of Fe, Co, Mn, etc., in an oil is not a reliable guide to the amount of catalyst present, because, apart from the varying activity of the metals, they are usually largely present as oxide, *e.g.* iron rust, in which form they are almost inactive.

5. A method is suggested of testing an oil for its sensitivity to metallic oxidising catalysts.

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2. C. H. Lea, *Proc. Roy. Soc.*, B, 1931, **108**, 175.

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A Colorimetric Method for the Determination of Minute Amounts of Mercury in Organic Matter

BY N. STRAFFORD, M.SC., F.I.C., AND P. F. WYATT

THE method described in the present communication was devised primarily for the determination of mercury in samples of grains, such as oats, wheat and barley, which had been treated with a seed disinfectant containing an organic mercurial. The amount of mercury present in the dressed seeds was known to be very small, a 5-g. sample containing at most less than 0·5 mg., and as a rule less than 0·2 mg. of mercury.

The determination of minute amounts of mercury has always been a difficult matter, particularly when the metal is associated with other heavy metals, or with large amounts of organic matter or inorganic salts. A comprehensive review of the existing published methods for the detection and determination of small amounts of mercury has been given by Cucuel.[1] We were, however, unable to find a published procedure which was applicable to the present problem.

At an early stage of the investigation it became evident that a satisfactory procedure would involve four distinct sets of operations:—(i) decomposition of the organic matter without loss of mercury; (ii) complete isolation of the mercury from the resulting solution; (iii) conversion of the isolated mercury compound into a form suited to the subsequent determination; (iv) actual determination of the mercury.

These four stages may most conveniently be considered in reverse order.

Experimental Work.

(*a*) DETERMINATION OF THE MERCURY.—Two colorimetric methods have been recommended for the quantitative determination of very small amounts of mercury: (A) the colloidal sulphide method,[2] and (B) the diphenylcarbazide method,[3] or the allied method using diphenylcarbazone.[4]

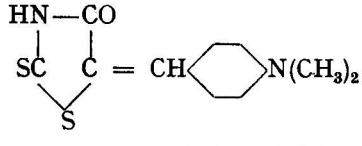
The sulphide method, apart from other disadvantages, was found to be insufficiently sensitive for the purpose in hand.

The reaction between diphenylcarbazide or diphenylcarbazone and mercury is extremely sensitive, but it is also extremely susceptible to interference. After careful investigation we finally decided against the use of these reagents. The following summary of our conclusions may be of interest:

The colour (an intense purple) can be developed in neutral, acetic acid, or sodium carbonate solution. Many salts partly or completely inhibit the colour formation, the shade as well as the intensity of the colour being affected. Halides and cyanides have the most marked effect, whilst nitrates, sulphates and ammonium salts, in all but the smallest amounts, greatly lower the sensitivity. Minute quantities of a large number of organic compounds have a similar effect, and even traces of free halogen or of hydrogen peroxide entirely prevent the formation of the mercury complex. It was also found that traces of a number of metals, such as copper, iron, lead, nickel, cobalt, cadmium and magnesium, give colours with both diphenylcarbazide and diphenylcarbazone under the conditions employed for the determination of mercury.[5] Although the mercury complex can be extracted quantitatively from an aqueous solution by means of certain organic solvents, of which benzene appears to be the most suitable, the coloured complexes formed by interfering metals are also extracted, so that this procedure does not make the method specific for mercury. It is thus evident that the quantitative determination of mercury by means of diphenylcarbazide or diphenylcarbazone is possible only after the mercury has been isolated completely from practically all other substances.

The only other sensitive reagent which has been used for the detection of mercury is *p*-dimethyl-amino-benzal-rhodanine, first proposed as a specific reagent for silver.[6] So far as we know, however, up to the present no use has been made of it for quantitative work. The conditions under which *p*-dimethyl-amino-benzal-rhodanine can be applied to the determination of mercury seemed much less stringent than those required for the diphenylcarbazide reaction, while its reaction with mercury is considerably more distinctive. For these reasons we finally adopted it for the quantitative determination.

p-Dimethyl-amino-benzal-rhodanine (*p*-dimethyl-amino-benzylidine-rhodanine)



gives with small amounts of mercuric ion a brick-red colour, the intensity of which is proportional to the amount of mercury present. With larger amounts of mercury a precipitate forms. The colour may be developed in neutral or acetic acid

solution, but is then masked to a large extent by the strong yellow colour of the excess of reagent. By adding a carefully controlled amount of nitric acid, the yellow colour of the excess of reagent may be discharged, whilst the colour due to the mercury persists. The mercury complex is preferably formed by adding the reagent to a dilute nitric acid solution of the mercury, of carefully adjusted acidity. Excess of nitric acid partly or completely prevents the formation of the complex. Whilst the reagent colour is just completely suppressed in $N/10$ nitric acid solution, this degree of acidity is not the best for satisfactory development of the mercury colour. Reduction of the acidity to $N/20$ results in more certain and more exactly reproducible formation of the red complex, especially with the smaller amounts of mercury ($<0{\cdot}05$ mg.). Under such conditions the solution is faintly tinted with reagent colour, but not enough to affect the colour gradation or the sensitivity of the method. The best gradation of colour is obtained with amounts of mercury ranging from 0·01 to 0·20 mg. in 100 ml. of solution.

Since the mercury complex is produced as a colloidal dispersion there is a marked tendency for precipitation to occur on standing. For this reason the colours in the test and standard solutions must be developed at the same time, and the comparison made as soon as the complex is fully formed. To prevent precipitation we at first used gum arabic as a protective colloid, until we found that this sometimes led to unreliable results.

In order that the colour may develop satisfactorily it is necessary to ensure that there is no local over-concentration of nitric acid before adding the reagent, *i.e.* the solution must first be diluted to the proper acidity and thoroughly mixed.

Sulphate or halogen ions, even in traces, prevent the satisfactory formation of the mercury complex; these ions must therefore be rigorously excluded from the final solution.

The only metals, other than mercury, which give a positive reaction with the reagent, under the conditions described, are silver, cuprous copper, gold, platinum and palladium. Cupric copper does not interfere.

As electrolysis between platinum electrodes forms an essential part of the final method, there would appear to be some danger of traces of platinum entering the final test solution and being determined as mercury; careful tests showed that this does not occur.

(*b*) Separation of the Mercury and Conversion into its final Form.—It is evident that whatever method of wet oxidation is used to decompose the organic matter, the resulting solution will contain all the inorganic constituents, such as silica, alumina, iron, magnesium, etc.; and since it is necessary to condense the volatile products to prevent loss of mercury, the solution will also be dilute and strongly acid. Modern workers agree that the best method of concentrating minute amounts of mercury from a dilute solution is by precipitation with hydrogen sulphide in the presence of a "carrier." Stock and his co-workers[7] have shown that it is possible to isolate as little as $0{\cdot}02\gamma$ of mercury from one litre of solution by this method. Booth, Schrieber and Zwick[8] adsorb on manganese hydroxide. The "carrier" most generally employed is copper sulphide,[9] and the use of this we adopted, since the presence of the copper also proved of assistance in the subsequent electrolysis (see later). Even in the presence of the copper sulphide

we noted a tendency for a little of the mercury sulphide to pass through the filter. This difficulty was completely overcome by precipitating the mercury and copper sulphides in the presence of a small amount of paper pulp and filtering through a paper-pulp filter. This ensures that the mercury is wholly retained by the filter, and renders the mercury sulphide more readily soluble in acid in the subsequent operation because of its well-dispersed condition.

For converting the mercury sulphide without loss into a form suitable for the colorimetric determination it was found best (1) to extract the mixed sulphides with carbon disulphide to remove free sulphur, and then decompose the sulphides and the paper pulp with small amounts of halogen-free concentrated sulphuric and nitric acids; and (2) to separate the mercury and copper from the resulting sulphuric acid solution by electrolysis. Removal of free sulphur is necessary, or mercury will be retained by the globule of sulphur which otherwise remains after the decomposition; and since the colorimetric determination cannot be carried out in a solution containing sulphate ions, the electrolytic separation is also necessary.

The presence of the copper previously added as "carrier" was found to facilitate the electrolytic separation of the mercury and to minimise the danger of loss of mercury by volatilisation from the cathode.

(*c*) DECOMPOSITION OF THE ORGANIC MATTER, AND PREPARATION OF THE RESULTING SOLUTION FOR THE SULPHIDE PRECIPITATION.—It is well known that when organic matter containing mercury is destroyed by a prolonged wet oxidation loss of mercury occurs by volatilisation. If, in addition, any free halogen or halogen ion is present, the losses become very large. Any method of decomposition must therefore provide for the effective scrubbing of the volatile products of the decomposition, so as to retain any mercury present in them. Further, since the mercury is to be isolated as sulphide from the resulting solution, only those oxidising agents (other than sulphuric acid) must be used which can readily be destroyed without loss of mercury. A mixture of conc. sulphuric acid and 100 volume (30 per cent.) hydrogen peroxide is suitable.

For condensing the mercury in the vapours from the decomposition flask it was found best to employ two traps in series, the first containing 5*M* sodium hydroxide solution, and the second containing water kept saturated with hydrogen sulphide, together with the small amount of copper sulphide (added as copper sulphate) and the paper pulp required as "carrier" for the mercury sulphide. Many alternative methods for retaining the volatilised mercury were tried, but virtually complete recovery was obtained only when the hydrogen sulphide trap was employed.

APPARATUS: (1) *Wet Oxidation.*—The apparatus is shown in the figure. The decomposition is carried out in the 100-ml. Kjeldahl flask, the neck of which carries a side-arm fitted with a glass-tapped funnel of about 15-ml. capacity. A condenser with a bulb of about 150-ml. capacity fits into the mouth of the flask by means of a carefully ground glass joint. The lower end of this condenser reaches nearly to the bottom of a trap consisting of a 250-ml. wide-mouthed conical flask containing 40 ml. of 5*M* sodium hydroxide solution, and closed by a tightly fitting 2-holed rubber stopper. A second condenser leads (as shown) from

this trap, its lower end reaching nearly to the bottom of a second trap consisting of a 100-ml. conical flask containing 25 ml. of water, 1·0 ml. of 0·04*M* copper sulphate solution, and 0·05 g. of paper pulp. The paper pulp is prepared by dividing a 12·5-cm. No. 44 Whatman paper into sixteen segments and thoroughly disintegrating one of these by breaking it up with a glass rod in a little boiling water.

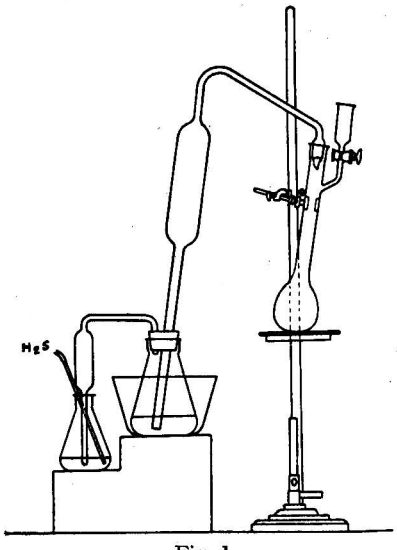


Fig. 1

(2) *Electrolysis.*—The electrolysis is carried out in an electrolysis vessel consisting of a flat-bottomed glass tube, 7 cm. high and 2 cm. internal diameter, with a small lip. The vessel is covered with a cover-glass with two holes blown in it, 1 cm. apart, through which pass the electrodes.

The cathode is a piece of platinum foil (5 cm. × 2 cm.) bent into semi-cylindrical form and supported by a stout platinum wire welded to its upper edge, and the anode is a platinum wire spiral of the same length as the cathode.

SPECIAL REAGENTS: (i) *p-Dimethyl-amino-benzal-rhodanine Reagent.*—Shake 0·04 g. of B.D.H. material ("spot test" reagent) with 200 ml. of alcohol, leave overnight, and filter.

(ii) *Standard Mercuric Nitrate Solution.*—Weigh accurately about 0·5 g. of clean, dry mercury, dissolve it in 5 ml. of conc. nitric acid and water, boil to remove nitrous fumes, and dilute to 250 ml. Dilute to a litre with water so that 1 ml. = 0·0001 g. Hg.

For the determination of smaller amounts of mercury further dilute this solution so that 1 ml. = 0·00001 g. Hg.

PROCEDURE: (1) *Decomposition of the Organic Matter.*—Weigh 5 to 8 g. of the seeds or other organic matter into the dry Kjeldahl flask, and connect the flask with the condensers and traps (as shown in Fig. 1), making sure that all joints are tight. Keep the first trap well cooled throughout the decomposition by means of a bath of ice and water. By means of a delivery tube drawn out into a fairly fine jet, pass through the liquid in the final trap a steady stream of hydrogen sulphide, at the rate of 2 to 4 bubbles per second. After five minutes run into the Kjeldahl flask through the tap funnel 10 to 15 ml. of conc. sulphuric acid, close the tap, and half-fill the funnel with 100 vol. (30 per cent.) hydrogen peroxide.

Heat the flask and its contents, supported on a sheet of asbestos in which is a small hole (1·5 cm. in diameter), by means of a small Bunsen flame, and run in at intervals 1 to 2 ml. of the hydrogen peroxide, adjusting the heating and the addition of peroxide so that no undue dilution of the sulphuric acid or frothing up into the neck of the flask occurs. Continue the decomposition (maintaining the steady stream of hydrogen sulphide through the small trap the whole time)

until a colourless solution is obtained, this usually being realised after about an hour's heating and the addition of 80 to 120 ml. of hydrogen peroxide.

When decomposition is complete, introduce slowly and cautiously through the funnel about 30 to 40 ml. of water, keeping the solution boiling to avoid a violent back-suction; although the addition of the water must be carefully controlled, there is no danger attaching to the operation, for the acid in the flask is already considerably diluted. Boil for 15 minutes to decompose the excess of peroxide, and then cool, opening the tap at intervals to equalise the pressure.

Discontinue the current of hydrogen sulphide and disconnect the apparatus. First transfer the alkaline contents of the larger trap to a 500-ml. beaker, cool in ice and water, and then add the acid liquid from the Kjeldahl flask very slowly with stirring, taking care to avoid loss through the vigorous evolution of sulphur dioxide and carbon dioxide which occurs. Wash out the Kjeldahl flask, the larger trap, and the large condenser with small amounts of water, and transfer the washings to the beaker, but placing apart the contents of the small trap and adding to them the washings from the small condenser.

(2) *Precipitation and Decomposition of the Mercury Sulphide.*—Add ammonium hydroxide (sp.gr. 0·880) to the contents of the beaker until a drop of the solution spotted on to Congo red paper gives only a faint greyish-black ring or spot. Then add a cold saturated solution of potassium permanganate, drop by drop, until the pink colour produced by the last drop fades out only slowly on stirring. Add the contents of the small trap, washing out the flask with cold water. Pass a fairly rapid current of hydrogen sulphide for twenty minutes through the cold solution, diluted to 300 to 400 ml., and containing also all solid matter remaining after the decomposition, and allow the solution to stand for a further twenty minutes.

Fit a small porcelain Gooch crucible (diameter of base 1·8 cm., height 2·3 cm.) with a pad consisting of 0·05 g. of paper pulp, prepared as described above, the pad being firmly pressed down with a flattened glass rod while suction is applied. Filter the solution through this crucible at the rate of 2 to 4 drops per second, wash out the beaker with hydrogen sulphide solution, and wipe its inner surface with a small piece of filter paper, which is then added to the contents of the crucible. Wash the filter with two 5-ml. portions of hydrogen sulphide solution, and then with four or five 2-ml. portions of acetone. The acetone dissolves most of any wax-like organic matter which has distilled over during the decomposition.

Dry the crucible and its contents for ten minutes in an oven maintained at 60° C. (± 5° C.) and extract the precipitate for half to one hour with carbon disulphide by Vortmann's method,[10] placing a small perforated porcelain disc in the crucible to prevent disturbance of the filter-pad. Then stand the crucible in a warm place for its contents to dry.

Detach the precipitate and paper from the crucible by means of a pointed wire and transfer it to a 100-ml. Kjeldahl flask, cleaning the inside of the crucible and the porcelain disc with a fragment of filter-paper. In all operations in which filter-paper is employed the amount used should be restricted to the bare minimum, or difficulty may be experienced with the subsequent decomposition.

Support the flask in an inclined position on a piece of asbestos board provided with a small hole (1·5 cm. diameter) and measure into the flask 1·0 ml. of conc.

sulphuric acid, followed by 1·0 ml. of conc. nitric acid. Heat gently over a low flame until the paper and precipitate are completely oxidised. If oxidation is difficult, a further small quantity of nitric acid (up to 1·0 ml.) may be added, the solution being heated until the sulphuric acid is slightly fuming.* More nitric acid, up to 1·0 ml., may be added, if necessary. Finally, add 2 ml. of water, boil gently until nitrous fumes disappear, and cool.

(iii) *The Electrolysis.*—Transfer the contents of the flask, including any insoluble silica derived from the sample under examination, to the electrolysis vessel, and wash out the flask with three 2-ml. portions of water. Render the solution slightly alkaline by adding ammonium hydroxide until the blue colour of the cuprammonium compound appears, the vessel and its contents being cooled in ice-water. Add *N* nitric acid until the solution is just acid and a further 1 to 2 ml. in excess, and adjust the volume to 20 ml. with water, mixing well during the dilution.

Electrolyse overnight (sixteen to twenty hours) at 3 to 4 volts, and with an initial current of about 0·05 amp. Wash the electrodes in the usual manner, with cold water, break the current, detach the cathode, and transfer it to a small test-tube (about 8·5 cm. long and 1·5 cm. diameter).

(iv) *The Colorimetric Determination.*—Add 5·0 ml. of *N* nitric acid from a pipette so that the acid runs over the electrode, and heat in a bath of boiling water for 15 minutes. Cool to room temperature, transfer the contents of the tube to a 100-ml. Nessler cylinder, and wash the test-tube and the electrode with cold distilled water.

Dilute the contents of the cylinder to 95 ml., mix the solution thoroughly, add 3·0 ml. of *p*-dimethyl-amino-benzal-rhodanine solution, adjust the volume to 100 ml. and mix.

Prepare standards for comparison as follows:—To suitable known amounts of standard mercuric nitrate solution in 100-ml. Nessler cylinders add 1·0 ml. of 0·04 *M* copper nitrate solution and 5 ml. of *N* nitric acid, dilute to 95 ml., mix, add 3·0 ml. of *p*-dimethyl-amino-benzal-rhodanine solution, dilute to 100 ml. and mix. Develop the colour in both test solution and standards simultaneously, allow them to stand five minutes, and compare.

If the total amount of mercury present exceeds 0·2 mg., it is advisable to carry out the colour comparison on an aliquot portion of the nitric acid solution, after adding sufficient *N* nitric acid to give a total of 5 ml.

ACCURACY OF THE METHOD.—Tests on the recovery of known amounts of mercury by the given method gave the following results:

(*a*) *Organic Matter Absent.*—Known amounts of standard mercury solution were originally present in a solution containing 10 ml. of conc. sulphuric and 5 ml. of conc. hydrochloric acid, and diluted to about 300 ml., a little hydrogen peroxide being also present in some instances.

Standard mercury solution (1 ml. = 0·0001 g. Hg.).

Added ml.	Recovered ml.	Added ml.	Recovered ml.	Added ml.	Recovered ml.
0·2	0·25	0·5	0·5	2·0	2·0
0·5	0·55	1·0	1·0	5·0	4·9
0·5	0·35	1·0	1·1	10·0	9·6

* Over-heating may cause loss of mercury by volatilisation.

(*b*) *Known Amounts of Standard Mercury Solution added to* 5—8 *g. of Mercury-free Oats.*—Standard mercury solution (1 ml. = 0·0001 g. Hg.).

Added ml.	Recovered ml.	Added ml.	Recovered ml.	Added ml.	Recovered ml.
0·5	0·5	1·0	0·75	3·0	2·6
0·5	0·5	2·0	1·8	3·0	2·9
1·0	0·95				

(*c*) *Weighed Amounts of an Organic Mercurial of known Mercury-content* (1·5 *per cent. Hg*), *added to* 5—8 *g. of Mercury-free Oats.*

Oats taken g.	Mercury added mg.	Mercury recovered mg.
5·0	0·396	0·360
5·0	0·211	0·200
5·0	0·129	0·105
7·5	0·190	0·180
7·5	0·047	0·038
7·5	0·025	0·020

It will thus be seen that, considering the very small amounts of mercury involved, the recovery is very satisfactory.

In conclusion, we desire to express our thanks to Imperial Chemical Industries, Limited, for permission to publish this communication.

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The Quantitative Separation of Aluminium and Beryllium

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Britton[1] examined this problem and described *inter alia* a satisfactory modification of Gmelin's method involving the hydrolytic decomposition of alkali beryllate. Later, Moser and Niessner[2] came to the conclusion that none of the processes described by Britton was of analytical value, and offered an alternative method. Newer methods have been described by other workers (*cf.* Schoeller[3]), and it was the purpose of the present investigation, not to add yet another method but to re-examine Britton's process, to determine whether Moser and Niessner's criticisms were justified, and, if so, to endeavour to ascertain wherein lay the inaccuracies and modify the process accordingly.

Stock solutions were made up and standardised as follows:—

Aluminium sulphate, free from beryllium (colour test, see below): Aluminium hydroxide was precipitated by addition of ammonium hydroxide, optimum conditions being obtained at *p*H 6·5 to 7·5 (Blum[4]), with the use of Chameleon No. 2 indicator (yellow-green within this range). After filtration, washing with ammonium chloride solution, drying, etc., in the usual way, the precipitate was ignited in a platinum crucible and weighed as aluminium oxide.

Beryllium sulphate, free from aluminium (colour test, see below): Beryllium hydroxide, $Be(OH)_2$, was precipitated by addition of excess of ammonium hydroxide, the mixture was boiled for 3 minutes before filtration, and, after ignition in the usual manner, the precipitate was weighed as BeO.

Initial Procedure.—The solution containing aluminium and beryllium sulphates (volume not greater than 50 ml.) was treated with 6 *N* sodium hydroxide solution until the precipitate first formed just re-dissolved, the mixture being agitated throughout the whole operation. After dilution to 400 ml. it was maintained at boiling-point for 40 minutes to complete the precipitation of $Be(OH)_2$; this was removed by filtration, ignited to BeO and weighed as such. The filtrate was acidified with hydrochloric acid, and the aluminium was separated as aluminium hydroxide by addition of ammonium chloride and ammonium hydroxide, ignited and weighed as aluminium oxide. The beryllium oxide was examined for aluminium by the aurine tricarboxylic acid reaction (*cf.* Clarke[5]), blank experiments with aluminium and beryllium having been carried out to ascertain the appropriate conditions. Similarly, the amount of BeO in the Al_2O_3 was determined colorimetrically (Duboscq colorimeter) by means of the quinalizarin reaction (Fischer[6]).

Series I.—In this series, approximately equal amounts of aluminium (in terms of Al_2O_3) and beryllium (in terms of BeO) were used. In experiments 1 to 4, the "initial procedure" described above was followed; thereafter, more care was taken with the aluminium precipitation, Blum's conditions (see above) being applied. In all these cases the colour tests yielded practically negative results, and the process appeared to be reliable enough to rank as a strictly quantitative procedure.

	Al_2O_3 (taken) g.	BeO (taken) g.	Al_2O_3 (found) g.	BeO (found) g.
1	0·3642	0·3564	0·3647	0·3570
2	0·3642	0·3564	0·3651	0·3575
3	0·2732	0·2673	0·2723	0·2661
4	0·2732	0·2673	0·2736	0·2665
5	0·4553	0·4455	0·4555	0·4460
6	0·4553	0·4455	0·4546	0·4451
7	0·5464	0·5346	0·5449	0·5336
8	0·5464	0·5346	0·5453	0·5332

SERIES II.—In this series an excess of aluminium was used in every instance. The results indicated that a considerable (varying) amount of $Al(OH)_3$ was co-precipitated with the $Be(OH)_2$; the colour tests verified this and showed the alumina to be free from beryllium.

	Al_2O_3 (taken) g.	BeO (taken) g.	Al_2O_3 (found) g.	BeO (found) g.
9	0·5464	0·1782	0·5351	0·1896
10	0·5464	0·1782	0·5204	0·2037
11	0·5464	0·0891	0·5372	0·0986
12	0·5464	0·0891	0·5268	0·0979
13	1·0928	0·1782	1·0391	0·2311
14	1·0928	0·1782	1·0353	0·2343

A slight modification was then introduced; instead of the mixture being maintained at boiling-point for 40 minutes after addition of sodium hydroxide, filtration was started after a very short period (2 to 3 minutes). A heated filter was used, and the filtration—always a slow one—lasted about 20 minutes. In these experiments the errors were considerably reduced, and the colour tests showed that this was not due simply to compensating incomplete precipitation of beryllium. Quantitative separations, however, could not be obtained.

	Al_2O_3 (taken) g.	BeO (taken) g.	Al_2O_3 (found) g.	BeO (found) g.
15	0·5464	0·1782	0·5386	0·1867
16	0·5464	0·1782	0·5391	0·1858
17	1·0928	0·1782	1·0512	0·2129
18	1·0928	0·1782	1·0469	0·2234

When double precipitation of the beryllium hydroxide was attempted, the results were again no better than with the method as initially standardised (*vide supra*).

	Al_2O_3 (taken) g.	BeO (taken) g.	Al_2O_3 (found) g.	BeO (found) g.
19	0·5464	0·1782	0·5364	0·1873
20	0·5464	0·1782	0·5350	0·1899

Finally, the following satisfactory procedure was adopted. If a preliminary determination indicated the presence of an excess of aluminium, a known quantity of beryllium salt (or solution) was added to make the amounts of Al_2O_3 and BeO approximately equal; the procedure outlined in Series I was then followed. Quantitative separation could thus be effected, the net amount of beryllium being determined by difference. The preliminary determination is sufficiently accurate

to indicate the amount of beryllium to be added, for, as is shown in Series III (below), the separation is quantitative also in the presence of an excess of beryllium, and so it is only necessary to avoid an excess of aluminium.

	Al_2O_3 (taken) g.	BeO (taken) g.	BeO (added) g.	Al_2O_3 (found) g.	BeO (gross) g.	BeO (nett) g.
21	0·5464	0·1782	0·3564	0·5457	0·5337	0·1773
22	0·5464	0·1782	0·3564	0·5448	0·5359	0·1795
23	0·3642	0·1782	0·1782	0·3651	0·3568	0·1786
24	0·3642	0·1782	0·1782	0·3639	0·3556	0·1774
25	0·5464	0·0891	0·4455	0·5452	0·5343	0·0888
26	0·5464	0·0891	0·4455	0·5461	0·5338	0·0883

SERIES III.—With an excess of beryllium over aluminium, the adopted procedure was found to be satisfactory:

	Al_2O_3 (taken) g.	BeO (taken) g.	Al_2O_3 (found) g.	BeO (found) g.
27	0·3642	0·5346	0·3637	0·5339
28	0·3642	0·5346	0·3645	0·5340
29	0·1821	0·5346	0·1829	0·5348
30	0·1821	0·5346	0·1816	0·5335
31	0·1821	0·8910	0·1809	0·8903
32	0·1821	0·8910	0·1814	0·8918

CONCLUSION.—The determinations described above confirm the conclusion that, so long as the amount of aluminium is not greatly in excess of the amount of beryllium, Britton's method (slightly modified) provides a quantitative separation of these two elements. Further, even with excess of aluminium, the process may be made quantitative by adding to the mixture a known amount of beryllium salt to adjust the balance.

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THE UNIVERSITY
ST. ANDREWS

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.

DIFFUSION METHODS IN QUALITATIVE MICRO-ANALYSIS. THE DETECTION OF ACETONE AND ALCOHOL IN BIOLOGICAL LIQUIDS

IN 1933 Conway introduced a simple form of vapour-diffusion unit for the determination of ammonia and urea in blood (Conway and Byrne, *Biochem. J.*, **27**, 420). The apparatus consists of a glass dish divided into an inner and an outer compartment, and sealed by a ground-glass plate. It is now on the market.* The principle is a general one, and can be applied to the detection and determination of a variety of volatile reactants, and has recently been used for the estimation of chloride and bromide in blood (Conway and Flood, *Biochem. J.*, **30**, 716).

We have been interested for some time past in the applications of the Conway unit to the detection of volatile pathological solutes in urine, blood, cerebro-spinal fluid and similar liquids. Among the more obvious substances capable of being detected in this way are: acetone, alcohol, ammonia, formaldehyde, formic acid, the halogens and the mercaptans. The success of the method depends on the use of a suitable reagent in the inner compartment and the preliminary treatment of the solution under examination to avoid the liberation of unwanted reactants.

I. THE MICRO-DETECTION OF ACETONE.—Two ml. of Nessler's reagent are placed in the inner compartment of the diffuser, and 2 to 3 ml. of the solution under examination are placed in the outer compartment. The solution must be slightly acid in order to prevent the escape of any ammonia that may be present.

The apparatus is closed, and kept at room temperature or incubated at 50° C. The presence of acetone is shown by the appearance of a pale yellow precipitate in the Nessler reagent. The method is extremely delicate. Acetone concentrations down to 0·01 per cent. give a reaction in less than a minute. At 0·002 per cent. the reaction is perceptible in about 5 minutes, and 0·0005 per cent. of acetone is detectable within an hour. When tested in this way, samples of normal urines yield no precipitate even after remaining for 12 to 24 hours. If, however, a specimen of normal urine be strongly acidified with concentrated hydrochloric acid before being placed in the diffuser, a precipitate appears within a couple of hours when incubated at 50° C. This we have found to be due to the liberation of volatile mercaptans—a contingency overlooked by some of the previous workers who have used Nessler's reagent as a test for acetone in urine (Denigès, *Précis de Chimie Analytique*, 6th ed., 1930, 213). For this reason, the urine should be acidified with a minimal quantity of dilute acid. When the solution under examination contains large quantities of acetone the precipitate in the Nessler reagent gradually re-dissolves in the excess of absorbed acetone vapour.

II. THE MICRO-DETECTION OF ALCOHOL.—Two ml. of a solution of 2 per cent. potassium chromate in nitric acid, previously diluted (1 : 2), are placed in the inner compartment, and 2 ml. of the liquid under examination are placed in the outer compartment of the apparatus. The diffuser is incubated at 50° C., and the presence of alcohol is shown by the development of a blue colour in the reagent (W. R. Fearon and D. M. Mitchell, ANALYST, 1932, **57**, 372).

* Obtainable from Messrs. A. Gallenkamp & Co., Ltd., Finsbury Square, London.

The nitro-chromic reaction is very delicate, and with a suitable dilution of the reagent will detect the alcohol in 0·1 ml. of a 0·025 per cent. solution after 24 hours' diffusion (Webb, *Sci. Proc. Roy. Dublin Soc.*, 1936, **21**, 281). Under these conditions, all specimens of normal urine examined showed the presence of minute quantities of alcohol.

The nitro-chromic reaction is, of course, not specific for ethyl alcohol, but responds to any compound containing the –CH.OH group. The reagent was originally shown by C. Ainsworth Mitchell to react to the presence of formaldehyde (ANALYST, 1896, **21**, 98), and for this reason the solution to be tested for alcohol should be treated previously with an excess of 5 per cent. silver nitrate and 20 per cent. sodium hydroxide to destroy any formaldehyde that may be present.

Where the Conway unit is not available, a tolerable substitute may be improvised by enclosing a watch-glass in a Petri dish.

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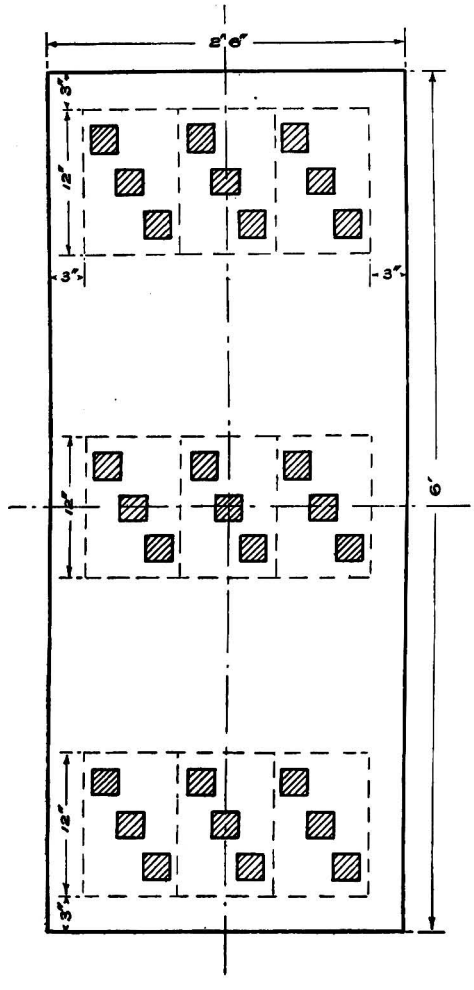
ZINC COATING ON GALVANISED IRON



THREE methods for the testing of zinc coating on galvanised iron are set out in the *Book of Standards of the American Society for Testing Materials*, 1930 Edition, p. 386, using:—(i) Hydrochloric acid with antimony trichloride; (ii) basic lead acetate; (iii) sulphuric acid and permanganate.

During the course of testing a sheet of galvanised iron by the hydrochloric acid and antimony trichloride method considerable variations were found in the amount of zinc coating at different parts of the sheet. As I was in doubt whether the method, which has been used in this laboratory for many years, was sufficiently trustworthy, I decided to investigate whether the variable results were due to the method or whether the zinc coating did actually vary so considerably.

In order to test this, two sheets of different brands of galvanised iron were obtained and sampled as shown on the accompanying diagram. Twenty-seven samples ($2\frac{1}{4}$ in. by $2\frac{1}{4}$ in.) were taken from each sheet and grouped in threes horizontally, nine samples being tested by each of the methods mentioned above.

The results obtained are set out in the following table, the figures being given in ozs. per sq. ft. of actual surface:

	Sheet A Methods			Sheet B Methods		
Sample	1	2	3	1	2	3
1_1	0·63	0·70	0·57	0·83	0·83	0·79
1_2	0·95	0·60	0·72	0·82	0·98	0·80
1_3	0·67	0·77	0·78	0·86	0·85	0·91
2_1	0·84	0·80	0·88	1·06	0·75	0·84
2_2	0·65	0·57	0·73	1·08	1·00	0·84
2_3	0·59	0·59	0·58	1·14	1·07	1·02
3_1	0·81	0·82	0·80	1·31	0·92	1·04
3_2	0·62	0·68	0·60	1·04	1·36	1·13
3_3	0·61	0·69	0·53	1·00	1·00	0·92
Average	0·71	0·69	0·69	1·02	0·97	0·92

From these results it is clearly seen that the zinc coating is not evenly distributed over the sheet. The three methods show approximately the same average. The hydrochloric acid and antimony trichloride method is undoubtedly the quickest, whilst the other two methods are slower to about the same extent.

Although the hydrochloric acid and antimony trichloride method is usually recommended as the most suitable for general routine testing, I am of the opinion that, on grounds both of accuracy and economy of chemicals, the sulphuric acid and permanganate method is preferable.

This method is as follows:—The weighed sample is completely immersed in 10 per cent. sulphuric acid, a piece of platinum being used as catalyst. After violent action ceases (about 20 minutes) the sample is removed, thoroughly washed, dried and re-weighed. The acid solution is then titrated with $N/10$ permanganate in order to determine the amount of dissolved iron. The difference in weight of the sample before and after the test, less the weight of iron dissolved, gives the true weight of the zinc coating.

J. A. D. Nash

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Official Appointments

The Minister of Health has approved the following appointments:—

Charles Adolphus Hackman and
Alexander Henry Mitchell Muter, as Public Analysts for the Borough of Colchester in place of W. F. Corfield (retired), July 20.

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.

METROPOLITAN BOROUGH OF HAMMERSMITH

Annual Report of the Public Analyst for the Year 1935

Copper in Vegetable Soup.—Two of six samples of tomato soup contained copper to the extent of 50 and 14 p.p.m., respectively. The occurrence of copper in tomato products seems to be more frequent than formerly. It may be due to the practice of spraying the growing tomato plant with copper fungicides, or more probably to preparation in copper vessels.

Chewing Gum Sweets.—Two samples of sweets, sold as "bubbly gums" and "Bobby gums," were free from injurious constituents. They were submitted for analysis following complaints that they had caused sore throat among children. They were sweets of the chewing gum type, and directions on the wrappers were given to the effect that, after all the sugary material had been chewed out of them, the residual gum could be used for blowing bubbles by pressing it against the teeth and blowing air into it. This has attracted children sufficiently to lead to a rather brisk sale of the sweets. According to my information the children pass the gum from one to another; hence, although there is nothing in the sweets themselves to cause sore throat, the disgusting and dangerous use to which they are put is quite sufficient to propagate much more serious disease.

White Iodine Cream.—A sample sold as "white iodine cream" bore a label stating that the content of potassium iodide was 2 per cent., whereas it was found to contain only 1 per cent.

F. W. Edwards

ROYAL BOROUGH OF KENSINGTON

Reports of the Public Analyst for the Fourth Quarter, 1935, and the First Quarter, 1936

Breakfast Cream.—Some reputable firms sell cream containing a rather low proportion of fat as "breakfast," "coffee," or "half-price" cream, and, presumably, no exception can be taken to this practice, but others sell such cream without declaration, and at the price of ordinary cream of normal fat-content. An attempt to regularise the position has been recently made in the Report of the Reorganisation Commission for Milk, in which the following standards were suggested:

Breakfast cream	..	12 per cent. of butter-fat.
Single cream	..	25 ,, ,, ,, ,,
Double cream	..	50 ,, ,, ,, ,,

The "breakfast" creams which I have so far examined have all contained as much fat as the Commission's "single" cream, so that it would appear that the suggested standard for the former is too low by half. The Commission seem to have no use for good Devonshire clotted cream with upwards of 60 per cent. of fat, or at least appear to have been at a loss for a name for it.

Preservatives in Canned Salmon.—Of three samples of canned smoked salmon, two were adulterated; one contained 700 p.p.m. of hydroxybenzoic acid and the other 660 p.p.m. The vendor of one sample was fined £2 and 10s. 6d. costs.

SPIRIT OF IODINE.—A sample sold under this name contained only 1·56 per cent. of iodine, and had been prepared with *iso*propyl alcohol as one of its constituents. Although exception could have been taken to the small proportion of iodine present, it was felt that as the name under which it was sold was not one of those used in the Pharmacopoeia or the Pharmaceutical Codex, a prosecution might have been inadvisable. F. W. EDWARDS

COUNTY PALATINE OF LANCASTER

ANNUAL REPORT OF THE COUNTY ANALYST FOR THE YEAR 1935

OF the 5189 samples examined during the year, 3304 were purchased formally. In the County of Lancaster it is now the usual practice to take formal samples of milk or spirits and (in the first instance) informal samples of other articles.

FOREIGN STARCH IN ARROWROOT.—A sample of arrowroot was found to contain about 1 per cent. of foreign starch. This starch resembled that of the sweet potato, *Ipomea batatas*, but may have been that of marble arrowroot, *Myrosma cannifolia*. The two starches are so nearly alike that it may not be possible to distinguish them with certainty when mixed with large quantities of other starches. Sweet potato is a foreign ingredient, but marble arrowroot, although not from a species of true arrowroot, sometimes occurs in the plantations and has been innocently mixed with the true arrowroot. The sample under discussion has been passed as genuine. During recent years the number of samples of arrowroot found to be adulterated has diminished, and it would appear that admixture with foreign starch, more frequent in the years 1926 to 1930, has now ceased.

STARCH IN MUSTARD.—Mustard, as sold in tins, often contains added starch in proportions of the order of 12 per cent., and a declaration of the fact is usually given. One sample, found to contain 50 per cent. of wheat starch, was sold in a tin bearing a label with the following words: "This mustard is sold as a mixture and is warranted to be of fine quality." This label was apparently designed to comply with Sec. 4 (1) of the Food and Drugs (Adulteration) Act, 1928, but a statement to the effect that a particular substance is a mixed article does not cover a case in which an ingredient is added fraudulently to increase its bulk, weight or measure. It might be considered to cover additions of starch up to the common amount of 12 per cent. (alleged to facilitate grinding, packing and storage), but might not be considered a sufficient declaration of the presence of a worthless diluent to the extent of one-half of the bulk of the article. On the attention of the manufacturers being called to the matter by the Clerk of the County Council, they agreed to alter the wording of the label to "This article is sold as a mixture of mustard and other ingredients."

SOYA-BEAN FLOUR IN SHREDDED SUET.—In 1931 the Council of the Society of Public Analysts expressed the opinion that, pending the establishment of a legally authorised standard, shredded suet should contain not less than 83 per cent. of fat. The average amount of fat found in commercial samples is about 88 per cent., the amounts found in the County Laboratory since 1928 varying from 77 to 100 per cent. One sample was found to contain 7 per cent. of coating and 93 per cent. of fat; it was labelled: "To prevent the shred from clogging a specially prepared flour is used for dusting." The sample was interesting in that the flour contained no starch (rice starch is the usual material), and that it had the characteristics of soya-bean flour.

CREAM OF TARTAR.—An informal and a formal sample each consisted of a mixture of sodium phosphate and maize starch, and were similar in composition to articles sold as cream of tartar substitute. Legal proceedings were not taken, since, at the time when the formal sample was purchased, the vendor made a declaration

as to its composition. Whilst it is doubtful whether such a declaration would have been made to an ordinary purchaser, it was felt that there was considerable doubt concerning the success of a prosecution. The vendor was cautioned.

WINE JELLY.—A sample sold as "wine jelly" was labelled: "This delicious . . . jelly contains the juice of luscious grapes and makes an ideal sweet." The jelly was free from all but the merest traces of alcohol, which were probably derived from the flavouring agents used. The Clerk of the County Council has previously been in correspondence with the manufacturers of this article, who agreed to alter their labels and advertisements. This has been done (*cf.* Annual Report for 1933, ANALYST, 1934, **59**, 482), but some of the statements even now do not appear to be free from objection. The matter is under consideration. G. D. ELSDON

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.

WATERED MILK CONTAINING MORE THAN 8·5 PER CENT. OF NON-FATTY SOLIDS

ON June 19th, at Leeds Police Court, two brothers, farmers in partnership, were summoned under Section 2 of the Food and Drugs (Adulteration) Act, 1928, for selling milk which was not of the nature, substance, and quality demanded, in that three samples, taken from different churns at a railway station in course of delivery to a dairy, contained 4·3, 3·0 and 3·2 per cent. of added water, respectively. A summons against a dairyman supplied by the defendants had previously been dismissed on production of warranty. Whereas, however, the dairy milk contained at least 7·7 per cent. of added water and 8·15 per cent. of non-fatty solids, the three farm milks contained 8·58, 8·61 and 8·75 per cent. of non-fatty solids, respectively, thus conforming to the Sale of Milk Regulations, 1901, even though they contained added water.

As a precautionary measure, therefore, alternative summonses were issued against the farmers under the Milk and Dairies (Amendment) Act, 1922, under Section 4 of which it is an offence to sell milk to which water has been added. The Stipendiary Magistrate, however, held that proceedings could rightly be instituted under the 1928 Act, as he considered that a purchaser was prejudiced if he was sold milk to which water had been added, even if the amount were insufficient to lower the non-fatty solids below 8·5 per cent.

Mr. Desmond Heap, prosecuting, stated that two appeal-to-cow samples contained 8·89 and 9·04 per cent. of non-fatty solids, respectively, the freezing-point in each case being −0·531° C.

Mr. C. H. Manley, M.A., F.I.C., Leeds City Analyst, giving evidence in support of his certificates, according to which the Hortvet freezing-points of the milks in question were −0·507°, −0·514°, and −0·513° C., respectively, stated that he estimated that there had been added to the three churns concerned 4, 2¾, and 4 pints of water, respectively.

As the defendants had been in business for 35 years without any previous conviction, the summonses were dismissed under the Probation of Offenders' Act on payment of £3 5s. 0d. costs, the prosecution's fees being remitted in respect of the alternative summonses issued under the 1922 Act.

FRUIT JUICE IN FRUIT WINES

ON March 13th a retail firm was summoned at Wolverhampton for selling raspberry and cherry wine not of the nature, substance and quality demanded.

Mr. Gore, prosecuting on behalf of the Corporation, said that the inspector had purchased, for 9½d. each, bottles of raspberry wine and cherry wine which did not contain the juice of the respective fruits, and he referred to a case in which the vendors of non-alcoholic blackcurrant wine were fined £15 for not putting the fruit juice into the wine.

Mr. F. G. D. Chalmers, B.Sc., F.I.C., the Borough Analyst, said that cherry wine should consist of the fermented juice of cherries, and that non-alcoholic cherry wine should contain at least 30 per cent. of cherry juice. Many of the recipes he had seen contained much more than that proportion.

In cross-examination he said that 1·53 per cent. of the cherry wine might have contained essence of cherries, and the raspberry wine might have contained 2·6 per cent. of raspberry essence. In both preparations there was a very large proportion of sugar and water. The cherry wine contained 23 per cent. of sugar, 74·45 per cent. of water, and 2·55 per cent. of other substances. He admitted that in all non-alcoholic wines there was a large percentage of sugar and water, and that there was a certain food value in the sugar.

The witness agreed that this type of wine had been sold all over the country for a very long time, but said that many vendors distinguished it by marking it "flavoured."

Mr. Batt, for the defence, suggested that the statement that 30 per cent. of fruit juice should be present in non-alcoholic wine was fantastic, as it would cause the preparation to ferment and become alcoholic. He challenged Mr. Chalmers to produce the published analyses and printed recipes on which he had based his opinion.

Mr. Chalmers produced copies of the analyses and recipes he had mentioned, but the Stipendiary, after inspecting them, pointed out that they referred to alcoholic wines.

Mr. J. F. Liverseege, F.I.C., said that there was no evidence of any cherry juice in the sample. It was composed of a syrup coloured with a coal-tar dye, and flavoured with an essence which was probably synthetic. It was impossible to make an artificial cherry juice, and, in his opinion, the public would expect to find a substantial proportion of cherry juice in a mixture labelled "cherry wine."

The secretary of the Birmingham and District Mineral Water Association said that, in his view, the label used by the defendants would deceive the public, and was not a fair description of the contents of the bottle. Of 21 sets of labels he had received from members of his Association, only one did not include the word "flavoured." In reply to Mr. Batt, he said that 30 per cent. of cherry juice would keep without fermenting and turning the wine alcoholic, if pasteurised.

A director of the manufacturing firm which had supplied the wine to the defendants said the preparation had been sold in large quantities all over the country. Fruit juice was not excluded because of the cost; the essence used was expensive.

Dr. T. H. Durrans, chief chemist to the firm who made the cherry essence used in the manufacture of the wine, said that the essence contained 25 per cent. of cherry juice and a substantial proportion of cherry concentrate. If more than ¼ oz. of essence in a gallon of liquid were used the flavour would be too strong.

The departmental manager of the firm who made the raspberry essence said that it was made from pure raspberries. One pound of the essence was equal to 7 or 8 lbs. of raspberries.

Dr. E. J. Parry, F.I.C., said that it was absolutely impossible to use 30 per cent. of fruit juice, as fermentation would set in soon after the bottle was opened, even if its contents had been pasteurised.

The Stipendiary, giving his decision on March 24th, said that the test was what the ordinary purchaser would expect to get. In his opinion they would expect to find something with a substantial amount of fruit juice. No juice at all, a very minute quantity, or some sort of essence, was not what would be expected. Two expert witnesses for the prosecution had said that they would expect to find 30 per cent. of fruit juice. At first he had regarded this figure as rather startling, though he later understood that this quantity referred only to alcoholic wines.

He had come to the conclusion that the summons had been substantiated, although he knew that the beverage was quite wholesome, and was, in fact, popular. The prosecution had stipulated a definite standard of fruit juice, but he did not propose to lay down any standard. It was comparable with pear drops. The juice was not included to create the wine, but to flavour the wine. Witnesses for the defence had said that if a large quantity of juice were added, it would ferment and possibly blow the corks out of the bottles. If a purchaser asked at a shop for a bottle of fruit juice he would probably find it difficult to get. To-day, this kind of thing was possibly made only at homes where wine was brewed. If, on the other hand, the purchaser thought that he was getting fruit juice, and did not get it, the bottles would not have been properly labelled.

The defendants had not been guilty of palming something on a customer when that customer could have got what he wanted by going to another place and asking for the same thing. In view of this he would order them to pay half the special costs (£4 4s. instead of £8 8s.), and impose a nominal fine of 40s.

Department of Scientific and Industrial Research

Water Pollution Research

SURVEY OF THE RIVER TEES*

In this report a detailed description is given of the results of a chemical and biological investigation of the estuary of the River Tees. This investigation, which occupied a period of about four years, formed part of a comprehensive survey of the whole of the river and its tributaries from its source on Cross Fell in the Pennines down to the sea.

The object of the survey was to obtain data regarding the effects of discharges of sewage and trade effluents on the river, and the extent to which these polluting liquids should be purified before discharge if serious pollution of the river water is to be avoided. In planning the work the aim was not merely to study the conditions affecting the River Tees, but to provide basic information of value in considering problems of river pollution in general. Sewage and trade effluents in a more or less crude condition are allowed to enter tidal waters from many districts on the banks of estuaries, and questions of treatment of the polluting wastes before discharge have become matters of some urgency.

According to the report just issued, the tidal section of the River Tees extends from High Worsall to the sea, a distance of 25 miles by river. From Yarm down to Stockton in this stretch the river flows between natural banks through country largely agricultural in character; the channel is not dredged and is little used by shipping. At low water between Yarm and Stockton the water is fresh, but at high springs salt water travels above Stockton to within one or two miles of Yarm. Below Stockton the estuary passes through a densely populated industrial area, and the channel, which is navigable, is dredged to ensure a minimum depth of

* Technical Paper, No. 5. Survey of the River Tees. Part II, The Estuary. H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Pp. 171. 1935. Price 9s. net.

about 12 ft. at low water. In the stretch of about seven miles from Stockton to Cargo Fleet below Middlesbrough numerous industrial effluents and untreated sewage from a population of about 280,000 are discharged. As a result, large numbers of migratory fish attempting to pass through the estuary are killed each year, especially in the spring, and the value of the salmon and sea trout fishery, which was formerly considerable, has greatly declined.

Although there is a general movement of the whole body of water up and down the estuary with each flood and ebb of the tide, hydrographical measurements and determinations of salinity have shown that there are additional movements. Fresh water from the upper rivers flows down the estuary mainly in the upper layers to reach the sea, and carries with it some salt water from the lower layers. At the same time salt water from the sea travels up the estuary in the lower layers. As a result of this circulatory system the water in the middle stretch of the estuary is stratified, and after heavy rain it is possible, at certain positions, to find nearly fresh water at the surface and almost undiluted sea-water at the bottom. Water entering the tidal reaches moves relatively slowly seawards, especially if the volume of fresh water from the upper river is small. It has been estimated that the average time taken for a body of water to travel through the estuary varies from about 2½ days in wet weather to about 6 days in dry summer weather. Substances carried in the upper layers reach the sea more rapidly and substances in the lower layers less rapidly than indicated by these average times.

As a result of the decomposition and oxidation of sewage and industrial effluents after discharge into the river, the water in the central part of the estuary is usually deficient in dissolved oxygen. The rate of oxidation of the polluting substances is greater at higher temperatures, and during hot summer months the concentration of dissolved oxygen may, on occasions, be as low as 5 per cent. of that in unpolluted river water; this concentration of oxygen is insufficient to support fish life. Various observations and experiments have indicated that of the reduction in the concentration of dissolved oxygen in the estuary of the River Tees, about 60 per cent. is due to the discharges of sewage, and about 40 per cent. to industrial effluents.

Near the mouth of the estuary the marine fauna and flora are varied and abundant, and at Yarm fresh-water animals and plants are numerous. In the central part of the estuary there are few marine or fresh-water organisms, particularly at Newport, 2 to 3 miles below Stockton. The region containing the smallest number of species is, in the Tees, coincident with the region of maximum pollution. With the object of assessing the relative effects of pollution and of changes in the salinity of the water due to tidal action, comparative surveys were made of the fauna and flora of the estuaries of the Tay in Scotland and the Tamar in Devon. The results showed that in all three estuaries the scarcity of marine and fresh-water organisms is due largely to unsuitable tidal conditions. In comparison with the other two estuaries, however, there are few, if any, fish living permanently in the central reaches of the Tees, and the numbers of certain shrimps are smaller.

Of the various industrial effluents discharged into the Tees, the most important are those from by-product coke works. The main toxic constituents of these effluents are cyanide and a group of phenolic substances known as tar acids. Approximately 2 tons of tar acids and nearly 1 ton of cyanide are contained in the average quantity of industrial effluent discharged each day. No other toxic substance enters the estuary in large quantities. Cyanide is much more toxic than tar acids, concentrations of 1 to 2 parts in 10 million parts of water being sufficient to kill fish in one hour. Systematic observations and experiments during periods when salmon and sea-trout smolts were migrating through the estuary to the sea proved definitely that cyanide, discharged as a constituent of effluents from coke-ovens, has been the main cause of the death of large numbers

of fish in the River Tees in recent years. Cyanide was frequently detected in the water of the estuary in concentrations sufficient to kill fish, and the gills of smolts picked up in a dying condition were brighter than normal in colour—a characteristic symptom of poisoning by cyanide. This conclusion was an important step forward in dealing with the problem, for, although various explanations had been suggested to account for the death of fish in the Tees, poisoning by cyanide had not previously been suspected.

Several methods of treatment of the effluents containing cyanide were examined. In experiments on a large scale by one method, 5,000 gallons of effluent per hour were treated with lime and with waste liquid from local galvanising works. The untreated effluent in 1 per cent. dilution killed fish in a few minutes, whereas the treated effluent in the same dilution was innocuous over a period of 24 hours. As a result of the work relating to effluents from coke-ovens, it has been concluded that the discharges of such effluents into the Tees could be greatly reduced in quantity, and possibly avoided, by modifications in the methods employed for cooling and washing coke-oven gas and by the utilisation of the waste liquids for quenching coke. It is understood that, as a result of the investigation, coke-oven installations to be erected in the future in the Tees area will be so designed that appreciable quantities of polluting liquids need not be discharged.

The pollution of the estuary by sewage could be reduced by treatment of the sewage in efficient purification works or by discharging it into the sea at a point some distance from the shore.

Commonwealth of Australia

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH

Division of Forest Products*

METHODS OF ANALYSIS OF PRESERVATIVE-TREATED TIMBERS

I. DETERMINATION OF ARSENIC

Two methods are described, one capable of determining 10 to 40 mg. of arsenic in 10 g. of wood, and the other capable of determining 0·5 mg. or less in 1 g. of wood.

In the first method (for 10 to 40 mg. of arsenic, *e.g.* occurring in the outside layers of treated timbers) the sample of karri (*Eucalyptus diversicolor*) is chiselled into thin sections, which are powdered by rasping or in an impact mill, in such a way that no dust is lost. Various methods of wet-oxidation and separation of the arsenic were investigated, and it was concluded that the procedure described in the First Report of the Sub-Committee of the Society of Public Analysts on The Determination of Poisonous Metals in Colouring Matters (ANALYST, 1930, **55**, 102), suitably modified for the larger quantities of sample involved, gave the most satisfactory results. The hydrazine sulphate may be replaced satisfactorily by 2 g. of cuprous chloride, if this is used immediately after the bottle is opened; after the reagent has been in contact with the laboratory atmosphere for some weeks its efficiency falls off considerably. The mixed sample is dried at 105° C., 10 g. are weighed into a 500-ml. Kjeldahl flask, and 2 ml. of water and 5 ml. of concentrated nitric acid are added for each g. of sample taken. When the reaction has subsided, the mixture is heated until brown fumes are no longer evolved, and 5 ml. of nitric acid per g. and 1·5 ml. of conc. sulphuric acid per g. are added without cooling.

* Reprint No. 29, 1936, pp. 10. By W. E. Cohen.

The S.P.A. method of wet combustion and separation of arsenious chloride by distillation is then followed, an apparatus for the distillation of 6 samples at a time being described; the only important point of difference is the introduction of a mixture of 7·5 g. of common salt, 1 g. of hydrazine sulphate and 1 g. of potassium bromide into the distillation flask. The distillate is collected in dilute nitric acid in an Erlenmeyer flask, and boiled almost to dryness on a hot plate, 100 ml. of water being then added and the boiling repeated. Finally, 10 ml. of sulphuric acid (1:1) and 50 ml. of water are added, and the boiling is repeated until white fumes begin to appear. The arsenic is then determined by diluting the solution to 150 ml., adding 1·5 g. of potassium iodide and boiling the mixture until most of the colour (due to the iodine) is destroyed, the final volume being over 50 ml. The cool mixture is diluted to 100 ml., the remainder of the colour is removed by means of a few drops of a 0·04 *N* sodium thiosulphate solution, and the resulting solution is made just alkaline to phenolphthalein with 33 per cent. sodium hydroxide solution, and then just acid with sulphuric acid (1 : 20). The volume is adjusted to 200 ml., 5 g. of sodium bicarbonate are added, and the solution is titrated with 0·04 *N* iodine solution (standardised against a standard solution of arsenic), 2 ml. of a 0·5 per cent. solution of starch being added near the end of the titration. Owing to the presence of sodium sulphate, the resulting starch iodide colour has a purple tinge.

For the determination of arsenic in the parts of the wood beyond the outside layers (*e.g.* 0·5 mg. or less), the samples are prepared in the same way, care being taken that they are reduced to a fine powder, and 1 g. of oven-dry material is taken for the wet-oxidation process. After the last addition of nitric acid 25 ml. of water are added and subsequently removed by boiling until white fumes appear, this procedure being repeated. The liquid is then diluted to exactly 200 ml., and a suitable aliquot portion (as determined by a trial test) is measured into a Douzard apparatus, together with a sufficient quantity of sulphuric acid (1 : 4 containing 100 g. of sodium chloride per litre) to make a total volume of 11·5 ml. This is followed by 2 ml. of a solution containing 84 g. of ferric ammonium sulphate and 10 ml. of the above-mentioned mixture of sulphuric acid and sodium chloride per litre, 1 ml. of a solution of 40 g. of stannous chloride in 100 ml. of conc. hydrochloric acid, and finally water, to make a total volume of 40 ml. A 1 per cent. solution of lead acetate (cleared with a few drops of 1 per cent. acetic acid) is placed in the purification bubblers, and a strip of filter-paper, which has been soaked in a 1 per cent. solution of mercuric bromide in alcohol and dried, is placed in the side-tube. Fresh arsenic-free zinc (15 g.), in rods 0·25 inch long and 0·25 inch in diameter, is added (10 g. being taken, if it has previously been used once). The apparatus is assembled, and after 1 hour the mercuric bromide paper is coated with paraffin wax and matched against a series of standard stains corresponding with 0·003 to 0·007 mg. of As_2O_3, the intensity and length of the stain both being taken into account. After the addition of 10 to 40 mg. of As_2O_3 to 10 g. of wood, the recovery was 98·0 to 99·6 per cent., whilst for additions of 0·0998 and 0·4992 mg. of As_2O_3 to 1 g. of wood the recoveries were 98 and 100, and 96 and 98 per cent., respectively. J. G.

Straits Settlements

REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

THE Government Analyst's Department (which is under the direction of Mr. M. Jamieson, B.Sc., A.I.C.) has four chemical laboratories in Singapore and one in Penang. The work comprises official work on behalf of other Government departments, and non-official work on behalf of the Government Departments of the Unfederated Malay States, Local Establishments of British Services and commercial firms. The total number of samples examined during the year was 3274. These were largely samples for revenue purposes and specimens for the police; the 27 food and drugs samples consisted of 25 samples of fresh milk, 1 of canned milk, and 1 of coffee. Two of the samples of milk were adulterated with buffalo milk, and 4 contained added water.

LEAD IN DISTILLED LIQUORS.—In July, 1935, in the course of the usual periodical examination for dissolved copper in distillery samsoo from Malacca, it was accidentally discovered that large quantities of lead were present in some of the liquors. Since then, all samples (299) have been examined for lead and copper, and attempts have been made to locate the source of contamination. Finally the copper was located in the receivers, which have now been replaced by wooden ones. Moreover, it was established that so-called pure tin used to coat the still, and the so-called pure block-tin piping of the condensers contained considerable quantities of lead.

METAL CONTAINERS FOR CHANDU.—An investigation was made into the practicability of making use of a new and cheap alloy of lead and antimony, in place of tin, for chandu containers. Unfortunately, tests on chandu stored in such tubes indicated absorption of dangerous amounts of antimony, and the new alloy could not be used.

IDENTIFICATION OF FIREARMS.—In connection with a Kreta Ayer shooting affray in February two lots of exhibits—(*a*) four automatic shells, (*b*) one shell and two bullets were examined. The shells in (*a*) were found to be fired from one weapon. The shell in (*b*) was found to be fired from a different weapon, and the two bullets to be fired from two weapons. Later in the year an automatic pistol and some rounds of ammunition were found on an arrested man. The pistol was deficient of a firing pin. A new one was fitted, and firing experiments were made with the seized ammunition. By means of comparative photographic enlargements (i) of the shells and (ii) of the bullet trace on thin lead foil, the automatic was identified as that from which the four shells and one of the bullets in the February shooting affray, had been fired.

IDENTIFICATION OF CARBON INK.—Two cases concerning seditious writing on walls were investigated. Writing scrapings from three lime-washed walls were identified as Chinese (carbon) ink. Stains on canvas shoes on an accused person and on a pipe-case were also demonstrated to be of carbon ink. The contents of two bottles were also found to be carbon ink. Such ink deposits, even when very small, give easily on extraction with water, a black suspension, the particles of which show Brownian movement. In this case evidence suggestive of identity among the inks was obtained in the flocculation of the suspensions with hot acid (owing, probably, to hydrolysis of a non-nitrogenous emulsifying agent) and in the high proportion of iron in the ash.

In the second case the circumstances and investigation were similar, but the amounts of material available were smaller, and recognition only, without experimental evidence as to identity of one deposit with another, was possible. No incriminating stains on the clothing were found.

TESTING OF GAS-MASKS.—One of the gas-masks for police use in cinema fires was tested to ascertain whether, at the end of the maker's guarantee (one year),

the canister required replacement or could be kept longer. Celluloid was burned in an iron drum to give a concentration of fumes comparable with that coming from 1000 feet of burning film in an operating room of 1000 cubic feet, and the gas produced was aspirated through the mask canister into (*a*) water and (*b*) oxalated blood. Little carbon dioxide and no carbon monoxide were detected in the effluent gas. The experiment was repeated, the emergent gas being passed through a wide-mouthed bottle containing a rat, without eliciting any symptoms of distress.

VETERINARY CASES.—Two specimens of crows' viscera and one specimen of duck's intestines were analysed for the Veterinary Department. No poison was found in the duck, but both specimens of viscera contained large amounts of formic acid. On investigation it was found that the birds had had access, for drinking purposes, to a drain into which the effluent from a rubber factory flowed.

In another case there was veterinary evidence that a dog had died of choking, but tobacco leaves were found in the stomach and nicotine was detected in the viscera. The conclusion was drawn that the animal had, accidentally or otherwise, consumed a portion of a Burma cheroot with its evening meal, and had been choked by its own vomit.

British Standards Institution

BRITISH STANDARD SPECIFICATIONS

THE following new specifications have been issued*:

No. 675.—1936. SUGAR FLASKS.

Two types of flask for use in sugar analysis are provided, *viz.* a doubly graduated type suitable for the analysis of sugar factory juices, and a singly graduated type, of greater accuracy, suitable for the polarisation of sugars. The first type is provided in three sizes, namely, 50-ml./55-ml., 100-ml./110-ml., and 200-ml./220-ml. capacity. The polarisation flask is provided in one size only, 100-ml.; the neck is enlarged above the graduation mark, but can be closed with the thumb for shaking.

No. 676.—1936. THREE SPECIAL FLASKS WITH GRADUATED NECKS.

The three flasks dealt with in this specification are primarily intended for the purposes indicated below:

45-ml. Flask with 5-ml. Scale.—This specification is to provide a standard flask for use in carrying out a polymerisation test as specified in British Standard Specifications Nos. 244 and 290 for turpentine.

150-ml. Flask with 10-ml. Scale.—This specification is to provide a standard flask for use in the official method of the Society of Public Analysts and Other Analytical Chemists for the determination of phenols in essential oils (THE ANALYST, 1928, **53**, 215).

200-ml. Flask with 25-ml. Scale.—This specification agrees in all particulars with the Standardisation of Tar Products Tests Committee's Tar Acids Flask, Schedule No. V.5, pp. 224 to 227 of "Standard Methods for Testing Tar and its Products," except that, to meet an expressed preference for this shape of bulb, a conical bulb has been specified instead of an approximately spherical one.

* Published by the British Standards Institution, 28, Victoria Street, London, S.W.1. Price of each 2s. net. Post free 2s. 2d.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Investigation of Fishy Flavour. W. L. Davies and E. Gill. (*J. Soc. Chem. Ind.*, 1936, **55**, 141–146T.)—Certain tentative conclusions as to the nature of "fishiness" have been formed, and these are supported by experimental work. Experiments show that increase of total nitrogen and of organically combined nitrogen in fish oils and ethereal extracts of fish products is accompanied by increase of fishiness and of brown colouring matter, and is associated with autoxidation. As much as 30 per cent. of the total nitrogen may be liberated and distilled from fishy oils after treatment with various reagents. A number of oils, particularly linseed, will enter into organic combination with nitrogen if kept for a period of weeks with a source of nitrogen such as betaine, casein, etc., or much more quickly if heated at 105° C. with trimethylamine oxide (considerable reduction to trimethylamine occurs). This combination is accompanied by a development of fishy odour. On the other hand, such odour is not produced by heating cholesterol or the unsaponifiable matter of vegetable or animal fats with trimethylamine oxide to 107° C., although the fatty acid fraction of linseed oil acts like the oil itself. An aqueous solution of maleic acid (at 100° C.) does not react with trimethylamine oxide, but in glycerol solution (at 120° to 130° C.) some reduction occurs, although there is no development of fishy flavour. Traces of peroxides, formaldehyde and tertiary nitrogen (either in the form of trimethylamine or its oxide or both), appear to be associated with fishiness, and positive reactions are given for formaldehyde and peroxides after fishy oils, their extracts or steam distillates have been treated with various reagents. D. G. H.

Betaine-content and Nitrogen Distribution of Beet Molasses and Other Beet By-products. W. L. Davies and H. C. Dowden. (*J. Soc. Chem. Ind.*, 1936, **55**, 175–179T.)—Beet molasses and molassed beet pulps were collected from 11 factories in Great Britain and analysed. The method used for determining betaine in molassed pulp was to shake 60 g. of the finely-ground pulp with 600 ml. of 5 per cent. milk of lime at 60° C. for 1 hour, to leave overnight, and to filter by suction. The volume of filtrate was determined, and for calculation the volume V was taken to correspond with V/10 g. of pulp. The calcium is precipitated by addition of sufficient sodium carbonate and filtered off; after acidification with sulphuric acid the filtrate is concentrated *in vacuo* to 100 ml. Total nitrogen is determined in an aliquot portion, and the remainder of the solution is treated in the same way as molasses, as follows:—Twenty g. of molasses are stirred with 10 ml. of water, and 100 ml. of 20 per cent. phosphotungstic acid in 5 per cent. sulphuric acid are added. After settling, the clear liquid is decanted through a hardened filter in a Buchner funnel, the precipitate is washed by decantation with 5 per cent. phosphotungstic acid in 2·5 per cent. sulphuric acid, 50 ml. in several washings being used; as much as possible of the precipitate is transferred to the filter with the last washing, and sucked dry. The precipitate is washed into

the original beaker, and the paper is moistened with baryta water and further washed. Powdered baryta is added and stirred in until permanent alkalinity is reached. The mixture is filtered, the barium salts washed, and the filtrate acidified with hydrochloric acid and evaporated *in vacuo* to 25 ml. Saturated solutions of sodium carbonate and mercuric acetate are added in turn until the precipitate is of a brick-red colour, when 150 ml. of alcohol (free from pyridine) are added, and the mixture is shaken and left overnight. The solution is filtered, acidified with hydrochloric acid, concentrated *in vacuo* to remove the alcohol, and made up to a convenient volume, and the total nitrogen is determined in an aliquot portion, excess of sodium sulphide being added to precipitate the mercury before distillation. Under these conditions, 1·75 mg. of betaine nitrogen escapes precipitation, and a correction of 0·009 is therefore added to the percentage found. The average betaine-content of molasses was found to be 5·4 per cent., and that of molassed pulp 1·8 per cent., the betaine accounting for approximately 38 per cent. of the soluble nitrogen in each material. Trimethylamine is present only to a very small extent—0·1 per cent. of the oxide in beet pulp, which is 5 to 8 times the amount in molasses. Ten lb. of molassed pulp contain the same amount of betaine as 60 lb. of fresh beet tops. D. G. H.

Analytical Values of Argentine Arachis Oils. C. G. Estrella and J. A. Duprat. (*Ind. y Quim.*, 1935, **14**, 14.)—The following results were obtained:

	Sp.gr. at 15°	Iodine value (Hübl)	Saponification value	Oleo-refractometer reading at 22° C.	Acidity (as oleic acid) Per cent.	Refractive index n_D^{15}	Turbidity temperature °C.
1*a*	0·9180	99·8	190·0	+12°	0·34	1·4715	+3°
2*a*	0·9178	101·0	189·6	+10°	0·36	1·4710	+2°
3*a*	0·9178	100·4	191·0	+12°	0·48	1·4715	+3°
4*a*	0·9178	99·6	190·0	+10°	0·38	1·4710	+2°
5*a*	0·9170	100·8	191·2	+10°	0·28	1·4710	+2°
6*a*	0·9175	99·5	190·1	+11°	0·41	1·4714	+4°
7*b*	0·9179	100·6	190·0	+12°	0·35	1·4715	+3°
8*b*	0·9175	101·2	189·0	+10°	0·40	1·4710	+2°
9*b*	0·9178	100·6	190·1	+10°	0·35	1·4710	+3°
10*c*	0·9176	99·8	190·7	+11°	0·40	1·4713	+3°
11*c*	0·9175	99·8	190·5	+12°	0·35	1·4715	+2°
12*c*	0·9179	100·8	190·8	+12°	0·41	1·4715	+3°

a Commercial refined oils, obtained from industrial establishments in the federal capital.
b Oils extracted in the laboratory with ether from seeds from the Province of Córdoba.
c Oils extracted in the laboratory with carbon tetrachloride from seeds from the Province Entre Ríos.

The purity of the oils was established by Bellier's method, modified by Mansfeld, Adler, Lüers and Evers (ANALYST, 1912, **37**, 487, 537). These values are similar to those generally obtained with arachis oil, with the exception of the oleo-refractometer reading, which is usually stated to vary from +3 to +8. E. M. P.

Chaulmoogra Oils. (*Bull. Imp. Inst.*, 1936, **34**, 145–154.)—Although the oil from *Hydnocarpus Kurzii*, Warb, is used in India, and that from *H. anthelmintica* in China and Siam, the most effective treatment for leprosy is by means of the oil

of *H. Wightiana*, Blume (an Indian species), and this oil is the only oil recognised in the British Pharmacopoeia. The cultivation of different species of *Hydnocarpus* is now being undertaken in many tropical parts of the Empire besides India, and samples of seeds of *H. Wightiana* from Nigeria, Malaya and Ceylon and of *H. anthelmintica* from Malay have been examined.

	H. Wightiana			*H. anthelmintica.*
	Nigeria	Ceylon	Malay	Malay
Average wt. of 100 seeds ..	118 g.	135 g.	—	—
Shell, per cent.	38·0	29·5	32·3	66·7
Kernel, ,, ,, ...	62·0	70·5	67·7	33·3
Oil, per cent. on dry kernel ..	64·3	65·9	68·0	63·0
Oil, sp.gr. at 25°/25° C. ..	0·9548	0·9557	0·9573	
$[\alpha]_D^{20°}$	+55·4°	+59·8°	+58·0°	
$n_D^{40°}$	1·4738	1·4740	1·4745	
M.p. ° C.	24·2	24·5	22·2	
Saponification value.. ..	202·2	201·1	205·1	
Iodine value (Wijs, 30 mins.)	98·2	99·1	98·9	
Acid value	1·2	1·4	0·3	

The *Wightiana* oil from Nigeria was further examined by fractionally distilling the free fatty acids, and the presence of hydnocarpic and chaulmoogric acids (the former in excess of the latter) was established; apart from a small amount of optically active liquid acid, possibly gorlic acid (*cf.* ANALYST, 1928, **53**, 604), no other fatty acids were isolated. The cold-drawn oil conformed to the requirements of the B.P. except on a point of minor importance, *viz.* the solubility in hot 90 per cent. alcohol—possibly owing to the low acidity. The Ceylon sample complied with the B.P. requirements, but the saponification value of the Malay sample was slightly high. D. G. H.

Quantitative Determination of Essential Oils in Solution in Alcohol. H. J. Van Giffen. (*Pharm. Weekblad*, 1936, **73**, 641–647.)—A volume of sample corresponding with about 200 mg. of actual oil (or about 8 g. if the oil-content is less than 1 per cent.) is weighed out exactly, by difference, and shaken in a 100-ml. separating funnel with 80 ml. of a 30 per cent. solution of ammonium sulphate. When the drops of oil have separated, the aqueous layer is poured through a small filter over which has been distributed 1 g. of "medicinal Norit" (other brands of adsorbent carbon have not always been found satisfactory). The separating funnel and filter are washed twice, each time with 5 ml. of the ammonium sulphate solution, and after addition of dilute sulphuric acid the filtrate may be used for the determination of alcohol (*cf. id.*, 1935, **72**, 1313). The filter and its contents are then extracted by shaking in the separating funnel with 15-ml. of ether in the presence of sufficient anhydrous sodium sulphate to absorb the water, and the extract is poured through a small filter into a tared 150-ml. flask, into which has been weighed exactly a quantity (about 0·5 g.) of liquid paraffin. The separating funnel and filter are washed 5 times, 5 ml. of ether being used each time, and the solvent is finally removed by distillation on the water-bath at a temperature not exceeding 40° C. The last traces are expelled by means of a stream of air, and the residue is finally placed in a (vacuum?) desiccator containing calcium oxide until

the loss in weight between two successive weighings does not exceed 1 to 2 mg. Comparison with the volumetric method described by Kaiser and Fürst (*cf. id.*, 1935, **72**, 1309; and *Deut. Apot. Ztg.*, 1935, 1734; 1936, 26) showed that the present method is more accurate, the calculated and experimental percentages of oil present in the following solutions being, respectively, 4·0 and 4·0 (in *Solut. Ammoniae spirit. anisata*); 10·0 and 9·9 (*Spiritus Menthae piperitae*); 0·3 and 0·3 (*Spirit. Lavandulae*); 0·34 and 0·325 (*Spirit. Rosmarini*); and 0·3 and 0·28 (*Spirit. Juniperi*).

J. G.

Determination of Citric Acid by Conversion into Acetone. K. Täufel and K. Schoierer. (*Z. Unters. Lebensm.*, 1936, **71**, 298–310.)—The separation of citric acid from the substances usually accompanying it by means of its acid quinine salt was found unsuitable for quantitative work, since a certain amount of the normal salt is formed. Precipitation by means of the bismuth nitrate and mannitol solution of Vanino and Hartl (*J. prakt. Chem.*, 1906, **74**, 142) was found to give accurate results when done in the presence of zinc carbonate and basic bismuth nitrate. Ten ml. of the solution freed from alcohol are placed in a 200- to 250-ml. flask with 10 ml. of water, or, if the amount of citric acid is less than 0·01 per cent., a suitable amount of the solution is evaporated to 20 ml. Powdered zinc carbonate (0·5 g.) and about 0·2 g. of powdered bismuth subnitrate are added, and the mixture is heated to boiling-point and placed on a boiling water-bath for 5 minutes during which time 5 ml. of 10 per cent. bismuth reagent (48·4 g. crystallised bismuth nitrate, 18·2 g. mannitol and 420 ml. water) are added in a rapid succession of drops. The liquid is quickly cooled and, after the lapse of about 15 minutes, filtered. The precipitate is washed twice with about 10 ml. of cold 0·1 per cent. bismuth reagent, and the filter and precipitate are replaced in the precipitation flask. Nine ml. of phosphoric acid (20 per cent. by vol.), 5 ml. of 25 per cent. acetic acid and 15 to 20 ml. of water are added, and the mixture is heated nearly to boiling-point and poured through a small filter. The previous filter is retained in the flask and washed by decantation at least four times with 10-ml. portions of hot water containing a drop of the phosphoric-acetic acid mixture, and finally with a little cold water. The filtrate is placed in the distillation flask and adjusted to the optimum *p*H value (Täufel and Mayr, *Z. anal. Chem.*, 1933, **93**, 1) by the addition of 5 ml. of 10 per cent. potassium hydroxide solution and 10 ml. of a phosphate buffer solution of *p*H value 1·9 (49·03 g. of 100 per cent. phosphoric acid and 60·08 g. potassium di-hydrogen phosphate dissolved in water and made up to 1 litre). Water is added, if necessary, until the flask is about half full. The distillation is carried out in a special apparatus, the essential features of which are that all connections are ground-in glass joints, the ground glass stopper of the distillation flask carries a dropping funnel, the receiver is interchangeable with the distillation flask, and a three-way tap allowing of communication with the atmosphere is interposed between the distillation flask and the vertical condenser. The receiver contains about 10 ml. of cold water, and the dropping funnel contains 0·05 per cent. potassium permanganate solution. When distillation begins, the permanganate solution is run in at the rate of not more than 1 drop per second, and brisk boiling is maintained until the appearance of

hydrated manganese dioxide indicates that oxidation is complete, whereupon the addition of permanganate is discontinued and distillation is allowed to proceed for another 15 minutes. The three-way tap is then opened. The condenser is washed down with a little water. Five ml. of a 1 to 2 per cent. solution of potassium permanganate and 5 ml. of 10 per cent. sulphuric acid are added to the distillate and, after the lapse of 20 to 25 minutes, the excess of permanganate is removed by means of saturated ferrous sulphate solution. The receiver is now used as the distillation flask, and 20 ml. of 10 per cent. potassium hydroxide solution are placed in the new receiver. The liquid is distilled for about 30 minutes. The alkaline distillate is treated with a suitable amount of $N/10$ iodine solution ($N/20$ if the amount of citric acid is less than 20 mg.), added drop by drop, with constant shaking. With small amounts of citric acid the reaction may take 2 to 3 hours; with larger amounts ½ to 1 hour. When the conversion of acetone into iodoform is complete the liquid is acidified with 20 ml. of 20 per cent. sulphuric acid, and the liberated iodine is titrated with standard thiosulphate solution, starch being used as indicator. The result of a blank determination is deducted. Each ml. of $N/10$ iodine used is equivalent to 3·5 mg. of citric acid containing 1 mol. of water of crystallisation.

A suitable method for the determination of citric acid consists in its separation by means of Denigès' reagent, and its subsequent photochemical oxidation to acetone by sunlight in the presence of ferric salts. Ten ml. of a solution containing 20 to 30 mg. of citric acid are placed in a 250-ml. flask with 10 ml. of a modified form of Denigès' reagent (30 g. of mercuric oxide are suspended in 100 ml. of water, 30 ml. of conc. sulphuric acid are added gradually, and the solution is made up to 1 litre), 10 ml. of 12 per cent. ferric ammonium sulphate solution, 2 ml. of 10 per cent. copper sulphate solution, 5 g. of solid potassium sulphate and 60 to 70 ml. of water. Five drops of 1 per cent. ferrous sulphate solution are then added. The flask is placed upon a white surface and exposed to sunlight or to the light of an ultra-violet lamp. As oxidation proceeds, the liquid becomes turbid, and finally a yellowish-white precipitate falls. To ascertain when oxidation is complete, another portion of the solution is exposed side by side with the first portion. This solution is filtered, and the filtrate re-exposed to the light. If no turbidity develops, oxidation is complete. The precipitate is filtered off and washed from the filter with a mixture of 5 ml. of 10 per cent. hydrochloric acid, 5 ml. of 20 per cent. sulphuric acid and 1 ml. of 10 per cent. copper sulphate solution, and the filter is rinsed at least four times with water. The liquid is placed in the distillation apparatus previously described and distilled into 25 ml. of 10 per cent. potassium hydroxide solution. The acetone is determined in the manner already described. A series of determinations of known quantities of pure citric acid varying from 0·5 to 10 mg. gave results varying between 100 and 100·7 per cent. A. O. J.

Santonin in English and Welsh Artemisias. J. Coutts. (*Pharm. J.*, 1936, **136**, 709–710.)—Samples of *Artemisia maritima* and *A. gallica* were collected from 27 localities in England and Wales, and the leaves, fine stems and (if present) flower-buds were air-dried and assayed for santonin by Coutts's method (*Quart.*

J. Pharm., 1932, **5**, 369). Santonin was present in all samples. In 20 batches of *A. maritima* the quantity varied from 0·5 to 0·8 per cent., in 5 of 12 batches, it exceeded 0·8, and in one sample from Essex it reached 0·96 per cent. Of 24 batches of *A. gallica*, 7 contained less than 0·5 per cent. of santonin, 12 between 0·5 and 0·8, 4 between 0·8 and 1 per cent., and 1 batch from Lincolnshire contained 1·24 per cent. It seems probable that *A. maritima* produces a slightly higher proportion of santonin than *A. gallica* at the same stage of growth and when growing in the same locality. As was found previously with Scottish plants, a seasonal variation in santonin-content was observed. Although evidence is incomplete that the soil, alone, or in conjunction with other factors, affects the production of santonin, it is probable that the salinity has a more or less pronounced effect. D. G. H.

Curaçao Aloes. P. A. Rowaan. (*Pharm. Weekblad*, 1936, **73**, 450–454.)—The properties of 3 authentic samples of Curaçao aloes (Bonaire, Curaçao and Aruba types, respectively) are compared in tabular form with those of 4 commercial samples, *viz.* 2 each of Cape aloes and Curaçao aloes; the data are discussed in the light of the requirements of the Dutch, British, American, French and German Pharmacopoeias. The results for the authentic and commercial Curaçao aloes and for the commercial Cape aloes were, respectively:—moisture, 8·3 to 9·7, 7·1 and 8·5, 9·5 and 10·8 per cent.; ash, 1·5 to 1·9, 1·3 and 1·6, 0·5 and 0·8 per cent.; the Bornträger reaction for anthraquinones: positive, positive and weakly positive; the Klunge-Stoeder reaction for *iso*barbaloïn, positive, positive, negative; solubility in 90 per cent. alcohol: good in all cases; solubility in ether: 0·4 to 0·6, 0·7 and 0·8, 0·5 and 0·7 per cent.; solubility in water: 66·8 to 74·2, 68·7 and 71·6, 60·2 and 60·5 per cent.; colorimetric evaluation (*cf.* P. van Wielen, *id.*, 1929, **66**, 877), 83 and 89, 67 and 100, 40 and 47. The results indicate that the colorimetric method and the reaction for *iso*barbaloïn may be used to identify Curaçao aloes. J. G.

Determination of Strychnine in Easton's Syrup. N. Evers and W. Smith. (*Pharm. J.*, 1936, **136**, 714–715.)—Although the British Pharmacopoeia method for the determination of strychnine in Easton's syrup is fairly satisfactory for freshly-made syrups, with syrups that have stood for some time difficulties arise in the washing of the alkaloidal residue. A new syrup was found to need five washings, but with an old syrup constant weight was not attained with seven washings. Also, the impure alkaloidal residue was not completely soluble in *N* hydrochloric acid. The suggested method is to carry out the assay as described as far as the stage at which the impure alkaloid is obtained. This is dissolved in 10 ml. of *N* hydrochloric acid, and the solution is filtered through a 9-cm. paper into a separator. The flask and filter-paper are washed with three further quantities of 5 ml. of *N* hydrochloric acid, and then with 25 ml. of a saturated solution of sodium chloride. The extraction of the filtered liquid is repeated by shaking with five successive quantities of 25 ml. of chloroform, and the assay is continued as in the B.P. process. D. G. H.

Measurement of the Proteolytic Activity of Pancreatic Preparations. N. Evers and W. Smith. (*Pharm. J.*, 1936, **136**, 714.)—Since the B.P. method was not found entirely satisfactory, the following modification of A. R. Smith's

method, based on the work of Sørensen, is suggested. Hammarsten's casein was used, as other specimens gave turbid solutions. Four g. of casein are dissolved in 90 ml. of water containing 3 ml. of *N* sodium hydroxide solution, the *p*H is adjusted to 8·7 (phenolphthalein as external indicator), and the solution is made up to 100 ml.. To 10 ml. of B.P. phosphate buffer solution at *p*H 7·0, one drop of a 0·1 per cent. solution of neutral red in 50 per cent. alcohol is added to make the neutral standard. Ten ml. of B.P. boric acid—potassium chloride—sodium hydroxide buffer solution at *p*H 8·7 are treated with 1 drop of neutral red solution (*vide supra*) and 3 drops of a 0·1 per cent. solution of phenolphthalein solution in 50 per cent. alcohol to form the alkaline standard. The required weight of the sample is triturated with a little chloroform water in a small mortar, washed into a 100-ml. flask and made up to volume with chloroform water, but not filtered. For the digestion 30 ml. of the casein solution and a definite volume of the enzyme solution are diluted to 100 ml., 50 ml. are removed as a control, and the remainder is rapidly heated to 55° C., kept at 55° C. for twenty minutes, and rapidly cooled. Two drops of neutral red solution are added to both liquids, followed by 0·1 *N* acid or alkali, until the colour matches the standard. Fifteen drops of 0·1 per cent. phenolphthalein solution and 10 ml. of formaldehyde solution (B.P.) are added to each liquid, and they are titrated with 0·1 *N* alkali until the colour matches the alkaline standard, the difference between the two titrations representing the amino-acids formed. The result is expressed as a volume of standard alkali for a definite weight of the enzyme preparation. Six samples of pancreatin, described as "B.P.," gave results varying from 12·5 to 47·0 ml. of *N* sodium hydroxide per 1 g. of preparation. A reasonable limit for pancreatin would be that 1 g. of a sample by the authors' method should require not less than 15 ml. of *N* sodium hydroxide solution, which is approximately equivalent to the present B.P. standard.

D. G. H.

Quantitative Determination of, and Molecular Weight Determination of Digitalis Glycosides by the Colorimetric Method. W. Neumann. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, **240**, 241–248.)—The method is based on the colour produced by the action of an alkaline picrate solution on the glycoside solution. The maximum extinction coefficient of the coloured mixture is determined in a Pulfrich photometer with the use of a 20-mm. cell and an S50 light-filter. A standard curve is given showing the relationship between the maximum extinction coefficient and the molar concentration. If the maximum extinction coefficient is determined for a given solution of unknown strength, the amount of glycoside present can be read from the curve, or, if the strength of the solution is known, the molecular weight of the substance can be calculated. The method is applicable to all glycosides having a $\Delta^{\beta,\gamma}$ lactone group in the molecule.

S. G. S.

Detection of Rhapontic Rhubarb in Galenical Rhubarb Preparations. S. K. Crews. (*Pharm. J.*, 1936, **136**, 720–721.)—The use of filtered ultra-violet radiation has made possible the detection of rhapontic varieties of rhubarb either alone or admixed with genuine official Chinese varieties, and the work has now been extended to include galenicals, those made with genuine unadulterated rhizome

showing no blue fluorescence. A wide-mouthed bottle of thin non-fluorescent glass of about 150-ml. capacity is nearly filled with water, and 0·5 ml. of the tincture or other liquid under examination is added. After mixing, a pledget of cotton, or other form of cellulose, about as large as a walnut, is added, and the bottle and contents are swirled round and allowed to stand for a few minutes. The cellulose is rinsed once or twice and examined under the quartz mercury-vapour lamp while still in water. A bright fluorescence on the cellulose indicates the presence of rhapontic rhubarb. A blank test should be made, as many forms of cellulose show some absorption. A modification of Wasicky's borax glycerin test enables the approximate proportion of Chinese rhizome present to be found. The sample, mixed with the special borax glycerin solution, is compared under the ultra-violet lamp with a set of standards prepared by mixing known amounts of official and rhapontic varieties in borax glycerin solution, but this solution must be treated with charcoal before addition of the rhubarb, to remove fluorescence due to glycerin. Results show that it is the practice of many manufacturers to use at least a proportion of rhapontic rhubarb in the manufacture of galenicals. D. G. H.

Detection and Determination of Preservatives. F. C. M. Jansen. (*Chem. Weekblad*, 1936, **33**, 1–8.)—A number of recently-introduced preservatives are discussed, with special reference to their use in mayonnaise and similar products. As a preliminary test (*cf.* Fischer and Stauder, ANALYST, 1931, **56**, 275), an extract of the sample in ether is shaken with alkali, which is then acidified with hydrochloric acid, and the acid liquid again extracted with ether. The new extract is dried over anhydrous sodium sulphate and evaporated below 40° C. It is then sublimed in an apparatus consisting of a long aluminium plate, which is heated at one end, while at intervals along it are depressions in which portions of the substance are placed and covered by a clock glass; a thermometer inserted near each depression registers the temperature at this point. The m.p. and sublimation-temperatures are given below. p-*Hydroxy-benzoic acid methyl ester* (Nipagin or Solbrol).—In warm acid solution this gives a red colour with Millon's reagent. It sublimes at about 70° C. (*cf.* Kofler and Kofler, *Mikrochem.*, 1931, **9**, 45), and if it is necessary to cool the receiver during this operation, the crystals concerned are the metastable modification (m.p. 110° C.); otherwise they are the stable type (m.p. 126° C.). Saponification with 5 ml. of a 2 per cent. solution of potassium hydroxide produces methyl alcohol, which may be separated by distillation and determined colorimetrically (*cf.* Von Fellenberg, *Biochem. Z.*, 1918, **85**, 69). A mixture of 3 ml. of the distillate, and 1 ml. each of a 5 per cent. solution of potassium permanganate and of a solution containing 21 ml. of 95 per cent. alcohol and 40 ml. of conc. sulphuric acid diluted to 200 ml., is shaken well. After a standing-period of exactly 2 minutes, 1 ml. each of 8 per cent. oxalic acid solution and conc. sulphuric acid, and 5 ml. of a solution prepared by dissolving 5 g. of fuchsine and 12 g of sodium sulphite in 100 ml. of *N* sulphuric acid and diluting to 1 litre (and stored in the dark) are added. The resulting colour may then be matched against that produced from a standard 1 per cent. solution of methyl alcohol, suitably diluted if necessary, in the same volume and under the same conditions. p-*Hydroxybenzoic acid propyl ester* (Nipasol) and *ethyl ester*.—The respective m.p.

are 97° and 116° C., and the sublimation-temperatures are both about 70° C. The separated dry ester is saponified by boiling for 1 hour with 2 ml. of 10 per cent. potassium hydroxide solution and 4 ml. of water under a reflux condenser, after which 4 ml. of liquid are separated by distillation, 2 ml. of this being mixed with 4 drops of 50 per cent. chromic acid solution in a Griebel apparatus (for producing crystals by the hanging-drop method). The fresh reagent (Fischer's reagent (ii), ANALYST, 1933, **58**, 569) is placed on the underside of the glass cover in such a way that crystals of the *p*-nitrophenyl hydrazone (which are insoluble in petroleum spirit) form in it when the chromic acid mixture is warmed. The crystals obtained from various aldehydes are described and their m.p. are tabulated. Nipacombin-A is the sodium compound of a 6 : 4 mixture of the above propyl and ethyl esters, and the m.p. of the corresponding mixture of acids is 95° C. p-*Hydroxybenzoic acid* (m.p. 213° to 214° C., sublimes at 135° C.)—Copper sulphate produces small, bright blue insoluble crystals when added to the warm acid, but the pyridine and copper sulphate reaction (see Steenhauer, *id.*, 1935, **60**, 577) should be used to distinguish this acid from salicylic, benzoic, cinnamic and anisic acids (*vide infra*). p-*Chlorobenzoic acid* (or its sodium salt, Microbin).—The m.p. is 236° C., and it sublimes at 95° C., corresponding figures for the *o*-compound being 142° and 75° C., respectively. A mixture of the specimen with 0·25 ml. of concentrated sulphuric acid and a crystal of potassium nitrate is heated on the water-bath for 20 minutes, 2 ml. of water and ammonia being then added, followed by 1 ml. of a 2 per cent. solution of hydroxylamine hydrate, when a positive reaction is indicated by a green colour at the junction of the liquids. *Cinnamic acid* (m.p. 133° C., sublimes at 90° C.).—An extract in acidified ether is made alkaline, evaporated, and re-extracted with acidified ether in the presence of a little alcohol to prevent emulsification. The extract is then washed 3 times with water and shaken with 0·33 *N* potassium hydroxide solution, ether being then removed from the separated water-layer by warming it. A 1 per cent. solution of potassium permanganate is added when the solution is cool, and benzaldehyde may then be recognised by its odour; the sensitiveness is 1 mg. of cinnamic acid, but this is lowered if the sodium salt is used. The purified acid may be determined by dissolving it in 0·1 *N* sodium hydroxide solution and titrating back with hydrochloric acid. Two sensitive reactions for benzaldehyde are described:—(i) One drop of a solution of phenol and 2 ml. of concentrated sulphuric acid are added, a hard red resinous mass being produced on warming. The mixture is cooled, diluted with 10 ml. of water, and made alkaline with 20 per cent. potassium hydroxide solution, when benzaldehyde gives a violet colour which may be extracted by shaking with acidified ether. (ii) To the oxidised liquid containing the benzaldehyde is added twice its volume of a solution of dimethylaniline in concentrated sulphuric acid, the mixture being then warmed to 150° C. and diluted with an equal volume of water. Malachite green separates on addition of potassium dichromate and sodium acetate. *Anisic acid* may be recognised by its reactions with ferric chloride and Millon's reagent, and by solubility tests. It is converted into *p*-hydroxy-benzoic acid and methyl iodide by the action of hydriodic acid, and may be separated from the former by extraction with chloroform in which anisic acid only is soluble. Preservatives may be removed from (*e.g.*) mayonnaise by shaking 50 g. successively

with one 100-ml. and two 75-ml. portions of ether in the presence of 2 drops of hydrochloric acid, the combined extracts being washed successively with one 100-ml. and two 75-ml. portions of water containing 5 ml. of 4 *N* sodium hydroxide solution in 100 ml. The aqueous layer is then treated with a 40 per cent. solution of calcium chloride, and after filtration the usual procedures of evaporation and extraction are followed (cf. *supra*), the final ethereal extract being dried over anhydrous sodium sulphate. It is evaporated at a temperature below 40° C., the residue is weighed, and the containing vessel is heated at a temperature above the sublimation-point of the preservative, when the loss in weight measures the amount of the latter originally present. The esters of *p*-hydroxy-benzoic acid may also be determined by the method of Weiss (ANALYST, 1930, **55**, 584). J. G.

Biochemical

Relative Values of Raw and Heated Milk. E. C. V. Mattick and J. Golding. (*Lancet*, 1936, **230**, 1132–1134.)—From the time of weaning, rats from the same litters were fed on biscuits made from flour and water, receiving in addition either raw milk, freshly sterilised milk or "kept" sterilised milk. Marked differences were observed between the groups. In the raw milk group litters from first matings were weaned to the seventh generation, but in the freshly sterilised milk group no third generation was weaned, and in the "kept" sterilised milk group no second generation was weaned. After the second generation the weight of the animals at a given age was lower than that of the original rats, and anaemia was probably present in the young of all animals on the experimental diet. Analyses of the bones of second generations indicated that the bones of animals on raw milk contained more ash and more calcium than those of animals having sterilised milk. No definite dental lesions were found, even in the seventh generation of animals receiving raw milk. S. G. S.

Deuterium as an Indicator in the Study of Intermediary Metabolism. Synthesis and Destruction of Fatty Acids in the Organism. R. Schoenheimer and D. Rittenberg. (*J. Biol. Chem.*, 1936, **114**, 381–396.)—Deuterium can be used for studying the synthesis and destruction of organic molecules in the living organism, and the rate of these reactions can be determined. Synthesis of fatty acids in mice on a diet rich in carbohydrates was followed by suddenly raising the deuterium content of the body fluids to 1·5 atoms per cent. (Atoms per cent. deuterium = per cent. deuterium atoms in the total hydrogen atoms of the water or organic compounds.) The deuterium-content of the fatty acids rose rapidly and reached a maximum in 6 to 8 days. Simultaneous destruction of fatty acids on the same diet was shown by another experiment, in which fatty acids containing deuterium, which had previously been deposited in the fat tissues, disappeared at about the same rate. The unsaturated acids synthesised by the mice were separated (*J. Biol. Chem.*, 1936, **113**, 505–510; Abst., ANALYST, 1936, **61**, 347) and azelaic acid was isolated as follows:—An amount of ozone equal to 1·5 times the theoretical amount, calculated from the iodine value, was passed into a solution of 2·5 g. of the unsaturated fatty acids in 100 ml. of acetic acid in $1\frac{3}{4}$ hours. After addition of 20 ml. of water and 2 g. of chromic trioxide, the solution

was allowed to stand overnight. The excess of chromium trioxide was reduced by adding methyl alcohol; most of the solvent was distilled off *in vacuo*, and the residue was extracted with ether. The aqueous solution was then extracted continuously with ether for 48 hours, and the two extracts were combined. The ether was distilled off and the residue treated with steam, in which azelaic acid is non-volatile. The azelaic acid was crystallised from a hot aqueous solution after treatment of the solution with charcoal and purified by re-crystallisation. This acid had the same deuterium concentration as the total fatty acids, proving that the results of the feeding experiments were not due to successive saturation and desaturation. The fatty acids of hens' eggs developing in a medium of heavy water do not take up deuterium into their molecules. Hence, the hydrogen atoms of these acids are not exchanged with those of water. Also, in eggs, appreciable hydrogenation of unsaturated fatty acids does not occur. In mice, there is a continuous conversion of carbohydrates into fatty acids under normal dietary conditions. The fat tissue is regarded as an energy buffer for the organism. E. B. D.

New Iodimetric Procedure for the Determination of Chloride in Small Amounts of Blood. G. A. D. Haslewood and E. J. King. (*Biochem. J.*, 1935, **30**, 902–905.)—The method is based on the liberation of iodine from potassium iodide by a soluble iodate formed when silver iodate is added to a chloride-containing liquid. There is always a "blank" titration due to the solubility of silver iodate in water. The silver iodate is prepared from silver nitrate (in very slight excess) and potassium iodate, 2 g. of the washed and dried precipitate being dissolved in 100 ml. of *N* ammonia solution. Both solid silver iodate and its ammoniacal solution decompose slightly on keeping, with the liberation of soluble iodate. Immediately before a series of determinations 5 ml. of the 2 per cent. ammoniacal silver iodate solution are acidified with 2 *N* sulphuric acid (5 ml.) and centrifuged. After removal of the supernatant liquid, the iodate is redissolved in 5 ml. of fresh *N* ammonia solution. This solution will last for at least one day. The chloride solution is treated with 1 ml. of the silver iodate solution and, after careful mixing, with 1 ml. of 2 *N* sulphuric acid. This mixture is shaken and filtered through a small paper of fine texture. Two ml. of the filtrate are treated with 1 ml. of 1 per cent. potassium iodide solution, and the liberated iodine is titrated with 0·005 *N* sodium thiosulphate solution, with starch as indicator. For the determination of the silver iodate "blanks," the quantities of iodate, as 0·1 *N* solution of potassium iodate, which should theoretically be set free according to the different chloride solutions used, are made up to 2 ml. with water. The resulting solution is treated as described above, and the treatment repeated with 1 ml. of *N* solution of ammonia instead of the silver iodate reagent. The difference between these titrations is the silver iodate "blank" for that particular chloride concentration. For values between 15 and 40 mg. of sodium chloride per 100 ml., the amount of chloride (as sodium chloride) is given by 5·275 (titre −0·65), and for amounts between 40 and 80 mg. per 100 ml. by 4·875 × titre. When the chloride in blood is to be determined, 2 ml. of a Somogyi (*J. Biol. Chem.*, 1930, **86**, 655) zinc hydroxide filtrate (1 in 10 dilution) are used as above. On a small scale the method is as follows:—The whole blood or plasma (0·2 ml.) is pipetted into

1·4 ml. of water (1·0 ml. for plasma), 0·2 ml. of 10 per cent. zinc sulphate solution and 0·2 ml. of 0·5 *N* caustic soda solution (0·4 ml. of each reagent for plasma) are added, and the whole is thoroughly mixed, and centrifuged. One ml. of the supernatant liquid (≡0·1 ml. of blood or plasma) is treated with 0·5 ml. of silver iodate reagent and, after mixing, with 0·5 ml. of 2 *N* sulphuric acid, and the mixture is shaken and filtered through a fine paper. To 1 ml. of the filtrate (≡0·05 ml. of blood or plasma) 1 ml. of 1 per cent. potassium iodide is added, and the liquid is titrated with 0·005 *N* sodium thiosulphate solution, with starch as an indicator. For titrations over 4·30 ml. the chloride (as mg. of NaCl per 100 ml.) is given by 97·5 × titre. For titrations less than 4·3 ml. the chloride is given by 105·5 (titre −0·65). The results compared favourably with those found by the gravimetric method. S. G. S.

Colorimetric Method of Determining Carnosine and Histidine with Bromine and with the Diazo-reagent. N. P. Meschkowa. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, **240**, 199–207.)—Carnosine cannot be determined with bromine in the presence of histidine, but the determination is possible if the diazo-reagent is used. The optimum concentration of reagent and of carnosine or histidine must be determined, and the volume of solution must be kept constant. Glycine, asparagine and uric acid affect the colour, but this effect can be overcome by using larger amounts of the reagent. Urea does not interfere with the reaction. When the correct amount of the diazo-reagent has been determined the colour intensity is the same for the same amounts of equimolecular solutions of carnosine and histidine. S. G. S.

Test for Thymine, with Observations on the Keto-Enolic Type of Diazo-Test. G. Hunter. (*Biochem. J.*, 1936, **30**, 745–749.)—The reagents required for the test are: a diazo-reagent made according to Koessler and Hanke (*J. Biol. Chem.*, 1919, **39**, 497),* a 1·1 per cent. solution of sodium carbonate, a 3 *N* solution of sodium hydroxide and a 20 per cent. aqueous solution of hydroxylamine hydrochloride. One ml. of the diazo-reagent is added to 2·5 ml. of the sodium carbonate solution. After 1 minute 0·5 ml. of a solution containing about 0·1 mg. of thymine is added. At the end of 5 minutes a faint yellow colour is perceptible and 1 ml. of the 3 *N* sodium hydroxide solution is then added. After another minute 1 drop of the hydroxylamine solution is added, and the whole is rapidly mixed. An intense red colour, which is stable for several hours, develops. A marked colour is produced with less than 0·01 mg. of thymine, but it is not given by 0·5 mg. of uracil or cytosine, although these produce yellow colours with the reagent and sodium carbonate. The term "keto-enolic type" of diazo-test is suggested to embrace the tests previously described as given by such substances as glucose, acetoacetic acid, acetaldehyde, acetone, tyrosine and now thymine, all of which are based on the same chemical principles. The

* The reagent is *p*-diazobenzenesulphonic acid, prepared by measuring into a 50-ml. flask 1·5 ml. of a solution of sulphanilic acid (4·5 g. dissolved in 45 ml. of hydrochloric acid of sp.gr. 1·19, and made up with water to 500 ml.) and 1·5 ml. of a nitrite solution (25 g. of 90 per cent. sodium nitrite in 500 ml.), placing the flask at once in an ice-bath for 5 mins., then adding 6 ml. of the nitrite solution, replacing in the bath for 5 minutes, and finally diluting to 50 ml. with water and storing in the ice-bath. It should be left for 15 minutes before use, and not used when more than a day old.

essentials for such a test appear to be the capacity for keto-enolic tautomerism in the substance to be coupled and the necessity for a reducing agent in the development of a significant colour from the product of coupling. S. G. S.

New Test for Bilirubin in Urine and its Use for Detection of Bilirubin in Normal Urine. H. N. Naumann. (*Biochem. J.*, 1936, **30**, 762–764.)—The test for bilirubin in urine consists in the adsorption of urinary pigments on a layer of talc and the production of blue bilicyanin by oxidation with a drop of Fouché's reagent or 10 per cent. nitric acid solution. A Buchner funnel (3·5 cm. in diameter) is fitted with an ordinary filter of 3 cm. diameter, and wetted with water. Five ml. of a 10 per cent. talc suspension in water are shaken well, poured on the filter, and dried by suction. Then 5 ml. of urine are poured on the talc layer, which after being dried again by suction appears as a yellow or orange disc. One drop of Fouché's reagent (25 g. of trichloroacetic acid, 10 ml. of 10 per cent. ferric chloride solution and 100 ml. of distilled water), or of 10 per cent. nitric acid is put in the middle of the talc disc and suction is applied. Even traces of bilirubin are indicated immediately by a distinct blue spot. The colour intensity increases for one or two hours and then fades slowly until, after 20 hours, only a faint grey is perceptible, unless the reaction has been very strong. This reaction is specific for bilirubin, for excessive amounts of indican react only after about 30 minutes. The limits of detectability of pure bilirubin dissolved in weak sodium hydroxide solution, diluted alcohol and urine freed from pre-formed bilirubin are 6·0, 0·8 and 0·9 p.p.m., respectively. From the last figure and by the use of a dilution technique, it is calculated that normal urine contains 0·3 mg. of bilirubin per 100 ml., and that the output is about 5 mg. per day. S. G. S.

Simple Micro-test for Acetone in Urine. J. F. Barrett. (*Biochem. J.*, 1936, **30**, 888–889.)—Acetone and acetoacetic acid may be detected by means of a precipitate produced with Nessler's reagent on boiling. The apparatus used is that described by Beaumont and Dodds (*Recent Advances in Medicine*, 1934, 7th Ed., p. 417) for the distillation of ammonia. The urine (0·2 ml.) and 3 ml. of salicylsulphonic acid solution (5 per cent. in 1 per cent. sodium sulphate solution) are placed in a "Monax" test-tube (8 × 1 in.). A porcelain chip is added, and the tube is closed with the trap-tube of the apparatus containing 0·5 ml. of diluted Nessler reagent (that of Koch and McMeekin, *J. Amer. Chem. Soc.*, 1924, **46**, 2066, diluted with an equal volume of water). The tube is placed on a sand-bath and heated steadily and strongly until steam begins to pass through the Nessler reagent. In the presence of 0·01 mg. of acetone or acetoacetic acid a small but definite creamy precipitate is formed. Volatile reducing substances, *e.g.* formaldehyde, interfere with the test, but this can be overcome by the addition of a drop of a 1 per cent. sodium hypochlorite solution to the Nessler solution, although this renders the test less sensitive. The test may also be applied to blood filtrates; 2 ml. of tungstic acid filtrate are used and treated as described. S. G. S.

Applications of a New Colour Reaction for Creatinine. S. R. Benedict and J. A. Behre. (*J. Biol. Chem.*, 1936, **114**, 515–532.)—Creatinine reacts with 3, 5-dinitrobenzoic acid to give a purplish-rose colour; the reaction is sufficiently

sensitive to be used for urine and blood filtrates. The coloured product is photosensitive and the colour fades after reaching a maximum development. In absence of direct sunlight, the rate of increase and of fading of the colour depends largely on the concentration of alkali present. Creatinine can be detected in a concentration of 0·01 mg. per 100 ml., and colorimetric readings can be taken in concentrations of about 0·2 mg. per 100 ml. Practically no blank colour is developed by the reagents under the given experimental conditions. Results obtained by the application of this reaction to various creatinine derivatives and to glycocyamidine and hydantoin are described. The method used for these is that described later under determination in blood filtrates, and results are compared with those obtained by the Jaffe reaction with picric acid carried out by (*a*) the original Folin method for blood (*J. Biol. Chem.*, 1914, **17**, 475), (*b*) the Folin-Wu blood method (*J. Biol. Chem.*, 1919, **38**, 81; Abst., ANALYST, 1920, **45**, 227). All the substances examined gave positive results with all the reagents or with none, but the results obtained with 2-benzylcreatine and with glycocyamidine were more distinctive than those given by the picrate methods, as regards both rate of reaction and colour produced.

Determination of Creatinine in Urine. Reagents.—(A) Solution of creatinine (0·1 per cent.) in *N*/10 hydrochloric acid. From this a standard solution (B) of 25 mg. of creatinine per 100 ml., is prepared daily by diluting (A) with water. (C) A 1 per cent. solution of 3, 5-dinitrobenzoic acid in 95 per cent. alcohol, kept in a brown glass-stoppered bottle. If this acid gives a colour with alkali, it should be purified by recrystallisation from glacial acetic acid; the method is described. (D) Six per cent. sodium hydroxide solution. *Method.*—The urine is diluted so that approximately 0·5 mg. of creatinine is contained in 1 to 2 ml. The dilutions are usually 1 : 1 and 1 : 4, for normal samples, and samples of high specific gravity, respectively; samples of sp.gr. less than 1·010 are usually undiluted. Two ml. of (B) and 1- and 2-ml. portions of diluted urine are measured into 3 small dry flasks. To the 1 ml. of urine 1 ml. of water is added; 3 ml. of (C) are then put into each flask, and exactly 1 ml. of (D) is added to each, as nearly simultaneously as possible, and the flasks are rotated. The solutions should not be exposed to direct sunlight. After 10 to 12 minutes, 10 ml. of distilled water are added rapidly to each flask by means of a burette; the time between the dilution of the contents of first and last flasks should not exceed 45 seconds. After mixing, colorimetric readings are made within 8 minutes of dilution by means of a photoelectric colorimeter (*cf.* Goudsmit and Summerson, *J. Biol. Chem.*, 1935, **111**, 421). The concentration of the unknown solution should be within 50 per cent. of that of the standard. Creatinine solutions of twice and one-half the value of standards containing either 0·5 mg. or 1·0 mg. of creatinine give readings within 95 to 98 per cent. of the correct values. The determination may be carried out in test-tubes graduated to 15 ml., with dilution to this volume.

Creatine and glucose give no colour by this method, but if large amounts of acetone or diacetic acid are present they should be removed. The accuracy of the method was tested by creatinine determinations on urine from which all creatinine had been removed by means of Lloyd's reagent, and to which a known amount of creatinine had then been added. To remove creatinine, 1 vol. of urine was diluted

with 1 vol. of oxalic acid, and water was added to make the same dilution as in the original determination. This solution was shaken for 4 to 5 minutes with 15 g. of Lloyd's reagent (fuller's earth) per 100 ml. of solution, the mixture was filtered, and the process repeated. After addition of 20 per cent. sodium carbonate, drop by drop, until precipitation was complete, the mixture was filtered; the filtrate was made neutral or slightly acid to litmus with hydrochloric acid. Determinations made on the filtrate after addition of creatinine gave slightly lower results for the new method than by the picrate method; the latter are too high by 2·5 to 5 per cent.

Determination in Blood Filtrates.—To 5 ml. of a 1 : 5 tungstomolybdic acid blood filtrate (Benedict, *J. Biol. Chem.*, 1931, **92**, 135), or ultra-filtrate, 1 ml. of a 5 per cent. alcoholic solution of re-crystallised dinitrobenzoic acid and 8 ml. of 5 per cent. sodium hydroxide are added. Solution (A) (see previous determination) is diluted with water to give the standards, and 5 ml. of the standard are treated in the same way as the filtrate. The standards are 0·1 to 0·5 mg. per 100 ml. No colour was given by the reagents alone, and pure creatinine in concentrations corresponding with 0·5 to 1·0 mg. per 100 ml. of blood gave enough colour for readings. The colour from the filtrates differs so much, in shade and stability, from that given by the creatinine, that the chromogenic substance in the filtrate cannot be determined as creatinine, and the results are interpreted as supporting the view that this substance is not creatinine. E. B. D.

Electrometric Titration of Insulin. Preparation and Properties of Iodinated Insulin. C. R. Harrington and A. Neuberger. (*Biochem. J.*, 1936, **30**, 809–820.)—The electrometric titration of crystalline insulin, in a specially designed apparatus for the use of the hydrogen electrode, is described, solutions in water and 80 per cent. alcohol being used. From the results obtained it is deduced that insulin has an acid-binding capacity of 43 ± 2 groups per mol. and a base-binding capacity of 60 to 70 groups per mol. Iodinated insulin has also been prepared, and it is shown that this differs from insulin only in that the tyrosine groups are substituted with iodine in the 3 : 5-positions. The iodinated product has been found to lose 90 to 95 per cent. of the physiological activity of the parent substance, but the partial removal of iodine by catalytic reduction is accompanied by an approximately proportional restoration of activity.

S. G. S.

Evidence concerning two Types of Plant Diastase. G. L. Teller. (*J. Biol. Chem.*, 1936, **114**, 425–430.)—Wheat and other grains were germinated for 96 hours, dried, and separated into bran, floury endosperm, and germ. Ungerminated grains of the same samples were similarly separated. Weighed amounts of each material (finely ground) were digested for 1 hour in 5 ml. of water at 20° C., and then with 50 ml. of starch paste, the *p*H of which was fixed by acetate buffer, at different temperatures. The action of the diastase was stopped by adding solutions of sulphuric acid and sodium tungstate, and the maltose formed was determined by means of potassium ferricyanide, as described by Blish and Sandstedt (*Cereal Chem.*, 1933, **10**, 189). It was found that germinating wheat, barley and rye, and also other plant products examined, contain two types of sugar-forming diastase. These have different activities in starch pastes of

different *p*H and at different temperatures. When both diastases are present, the amount of maltose produced at a given *p*H and temperature is a resultant of the combined action of the two. At 60° C. the ratio of the maltose produced in a paste of *p*H near 4·5 to that in a paste of *p*H near 6·2 is greater than 1 for the diastase characteristic of wheat flour, reserve diastase (A). For the other diastase, vegetative diastase (B), which predominates in the bran of germinating cereals, this ratio is less than 1. For both diastases, if the reaction is carried out at different temperatures, the maltose ratio for *p*H 4·5 and *p*H 6·2 tends to approach 1 as the temperature is lowered. The ratio obtained experimentally at 60° C. for a given plant product is used as a means of indicating which type predominates in this product. In wheat grains and the sweet potato (A) and (B) are both present, not only in the germinating grains, but also at the earliest stages of formation of the immature seeds. E. B. D.

Water-soluble *B*-vitamins. Flavin and Vitamin B_6 in Cereals. A. M. Copping. (*Biochem. J.*, 1936, **30**, 849–856.)—The use of diets of the Bourquin and Sherman type, containing extracts of wheat or maize, or their milled products, or the unextracted whole cereals, for experiments on rats, has shown that wheat and maize are good sources of vitamin B_6, one-third to one-quarter of which can be extracted by cold 80 per cent. alcohol. Wheat contains more flavin than maize, but this substance is not extracted by cold 80 per cent. alcohol. In both of these cereals more vitamin B_6 is contained in the germ and integuments than in the endosperm. An indication was also obtained that a deleterious substance, extractable by 80 per cent. alcohol, is present in maize and maize extracts. S. G. S.

Occurrence and Chemical Nature of Vitamin *K*. H. Dam and F. Schønheyder. (*Biochem. J.*, 1935, **30**, 897–901.)—Vitamin *K*, which is thermostable, is found in fairly large amounts in green vegetables, and certain mammalian livers contain appreciable quantities. The activity of hog-liver fat is reduced to about one-third of its original value by cold saponification, and completely destroyed by hot saponification. Solvents, such as alcohol and acetone, extract more vitamin *K* from alfalfa than is indicated by direct feeding of the vegetable, and this is explained by assuming that there is incomplete extraction in the alimentary tract. During attempts to prepare a concentrate it was found that inactive material could be removed from a petroleum spirit solution by means of 90 per cent. methyl alcohol. When calcium carbonate or sucrose was used as an adsorbent, a concentrate of 600,000 to 1,000,000 units per g. could be obtained, but alumina adsorbed the vitamin so firmly that it could not be eluted by a mixture of ethyl alcohol and benzene. S. G. S.

Quantitative Determination of Vitamin *K*. F. Schønheyder. (*Biochem. J.*, 1936, **30**, 890–896.)—If food, containing sufficient vitamin *K*, is fed to an animal suffering from a deficiency of this vitamin, the clotting-time becomes normal in three days, and therefore the curative method is the best one for its determination. The blood plasma is characterised by the concentration of the clotting agent which, upon the addition of 1 to 5 drops of 50 per cent. plasma, would cause the latter to

clot in 180 seconds at 40° C. The relation between the concentration required to cause the plasma from a diseased animal to clot in 180 seconds and that required for a normal plasma is a quantitative measure of the degree of sickness of the animal. This relation, multiplied by 10, is called the *S* value of the animal. A unit of vitamin *K* is defined as the smallest daily dose of the test substance (which should be administered in the form of a tablet) per g. of chicken, given for 3 days, which reduces the *S* value from over 1500 to 10. S. G. S.

Bacteriological

Specific Curves of Bacterial Growth recorded Photometrically. M. Faguet. (*Ann. Fermentations*, 1935, **6**, 348–360.)—The author describes an original method for observing bacterial growth by measuring and recording the light diffused by cultures of micro-organisms when a ray of light is directed upon a tube containing them. He refers to the formula worked out by Lord Rayleigh and modified by Schuster, which expresses the relationship between the intensity of a beam of light directed upon a suspension of fine particles and that of the diffused light emerging at an angle of θ degrees from the incident beam, from which it is seen that the number of particles per unit volume is a function of the ratio of intensities of the incident to the diffused light. This law, he remarks, only holds when the particles in suspension can be considered small in comparison with the wave-length of the light employed, and is, therefore, not strictly applicable to bacteria, but the measurement of the diffused light can be used to estimate roughly and record the number of bacteria present and to trace their growth.

The apparatus he uses consists of the following:—An electric incandescent lamp with compact filament and heated by a constant current at constant e.m.f. as the source of illumination (0·3 amp. at 12 volts); a condenser adjusted to direct a beam of parallel rays upon a screen-filter of coloured gelatin; a second condenser to converge the rays emerging from the screen upon a test-tube (23 mm. in diameter) containing the culture; a photo-electric cell placed close to, and at the side of, the test-tube at right angles to the axis of the incident rays; a reflecting galvanometer to which the photo-electric cell is relayed, the deflections of which are traced on photographic paper mounted on a clock-worked drum. A suitable diaphragm shields the photo-electric cell from direct rays. The light focused upon the tube is almost monochromatic, of medium wave-length (λ=about 0·680μ), and the beam strikes the walls of the test-tube almost normally, thus reducing loss and reflection to a minimum.

The calibration of the galvanometer scale in terms of intensity of diffused light is carried out as follows:—A stable turbid medium is placed in position in the test-tube and the galvanometer reading taken. The photo-electric cell is then placed in the direct beam of light, and the galvanometer is brought back to the same reading by the use of a photometric prism ("coin photométrique"). If D is the optical density of the prism, we have:

$$D = \log_{10} \frac{\text{intensity of incident light}}{\text{intensity of diffused light}}$$

Thus, if D = 2, the culture diffuses in the direction of the photo-electric cell

1/100th part of the energy it receives, and if D = 3, 1/1000th part. A logarithmic graduation is thus obtained.

The whole apparatus, except the galvanometer, is placed in an electrically-heated bath at 35° to 40° C., so that the growth of bacteria, as measured by the turbidity of the culture, can be recorded automatically. Curves are given, showing the growth in carefully standardised media, of *B. coli*, *B. typhosus*, *B. paratyphosus A*, *B. mucosus capsulatus* (the pneumobacillus of Friedländer), and *Staphylococcus aureus*. Comparative curves are also given showing the growth of the same bacteria under the inhibiting influence of a filtrate of broth containing a growth of lactic acid bacilli, and under the greater inhibiting influence of a filtrate of broth containing autolysed lactic acid bacilli. It is claimed that, with standard seeding and with the standard media employed, these curves are sufficiently characteristic to distinguish the micro-organisms investigated, one from another, and that they are reproducible to the extent of being superposable. D. R. W.

Agricultural

Accuracy of the Determination of Lead and Arsenic on Apples. D. E. H. Frear and W. S. Hodgkiss. (*J. Agric. Res.*, 1936, **52**, 639–644.)—Errors incident to these determinations are due to (*a*) errors in sampling, and (*b*) errors in the technique and standardisation of the actual method. Lead is determined by the photo-electric method of Frear and Haley (*Pa. Agr. Expt. Sta. Bull.*, 1934, 304). Light from an electric bulb, regulated by a suitable rheostat, is directed through a cylindrical glass tube containing the solution to be analysed on to the surface of a No. 594 Weston photronic cell connected directly with a 200 microamp. microammeter. The rheostat is adjusted so that the microammeter records its maximum value, and the lead in the solution is then precipitated by addition of sodium sulphide solution. The resulting colour reduces the amount of light falling on the cell by an amount which is measured by the microammeter, this being calibrated in terms of known quantities of lead. The Gutzeit test is used for arsenic determinations (A.O.A.C., *Official and Tentative Methods of Analysis*, 1930, p. 593), and its accuracy has been discussed fully elsewhere (Neller, ANALYST, 1929, **54**, 618; Barnes and Murray, *Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 29). The probable error of the lead determination considered alone was found to be ±0·0028 mg. for samples containing 0·4000 mg. of lead. For the entire procedure (*i.e.* sampling and determination) and with a mean amount of lead present of 0·02246 grain per lb. of fruit, the average difference between duplicates was 0·00304 grain per lb.; corresponding figures (as As_2O_3) for the entire arsenic determinations were 0·00944 and 0·00140 grain per lb., respectively. The lead determination is slightly more accurate than the Gutzeit test, even when allowance is made for sampling errors, which are much the same in both instances. Thus, the average deviations of individual determinations from the mean of duplicate determinations were 6·8 and 7·4 per cent., respectively; the ratio Pb:As_2O_3 in the mean values was 2·37:1, and the ratio average deviation Pb: average deviation As_2O_3 was 2·15:1. Lead present on the surface of apples as spray residue may

combine chemically with the waxy coating of the apple, whilst, apparently, arsenic does not. These data are based on the mean values of 164 samples of apples analysed in duplicate. J. G.

Colorimetric Determination of Phosphoric Acid in Fertilisers. K. C. Scheel. (*Z. anal. Chem.*, 1936, **105**, 256–269.)—For the rapid determination of phosphoric acid the author uses the method of Fiske and Subbarow (Abst., ANALYST, 1926, **51**, 205) with *p*-methylaminophenol sulphate as a reducing agent, and measures the depth of colour in a Pulfrich photometer (Zeiss). The following solutions are required: (1) 1 g. of the reducing agent, 5 g. of sodium sulphite, and 150 g. of sodium bisulphite, dissolved in 500 ml. of water. The filtered solution, kept in a well-stoppered bottle, is quite stable. (2) Ammonium molybdate (50 g.), dissolved in 500 ml. of 10 *N* sulphuric acid, diluted to 1 litre and filtered. (3) 1 litre of 5 *N* sodium hydroxide solution, adjusted against the acid used for the molybdate solution, is neutralised with acetic acid, filtered, and diluted to 2 litres. The solution is added as a stabiliser. (4) Standard solution: 1·9167 g. of monopotassium phosphate, dried over sulphuric acid, is dissolved in 1 litre of water, a few drops of chloroform being added (1 ml. = 0·0010 g. P_2O_5). The strength is checked gravimetrically.

Part of the standard solution is diluted to half-strength. Portions of solution containing 0·5, 1·0, 1·5, 2·0, and 2·5 mg. P_2O_5 are measured with 1-ml. precision pipette into 100-ml. flasks, diluted to about 50 ml., and treated with 5 ml. of reducing solution and 10 ml. of molybdate solution. After 10 minutes, 20 ml. of acetate solution are added, and the whole is diluted to 100 ml. The colour measurement is carried out with a 10-mm.-layer and an S72 filter, the mean of 5 to 6 readings being taken. If the curve constructed on that basis does not cut the co-ordinate system at zero, either the zero-point of the instrument has not been correctly adjusted, or the reagents contain impurities, which must be allowed for by a blank.

The determination of phosphoric acid in fertilisers is carried out in the same manner, the weight taken being calculated to yield 1 to 2·5 mg. P_2O_5 in 1 ml. In the determination of total phosphoric acid, separation of the silica is unnecessary, the sample being decomposed with strong sulphuric acid. A series of 10 analyses can be carried out in 1½ hours. The error is given as ±0·6 per cent., about half of which is incurred in the colour measurement. W. R. S.

Organic

Use of S-Benzyl Thiuronium Chloride for the Isolation and Identification of Organic Acids. J. J. Donleavy. (*J. Amer. Chem. Soc.*, 1936, **58**, 1004–1005.)—The constitution of the reagent, which was established by Werner (*J. Chem. Soc.*, 1890, **57**, 285), and confirmed by Lecher *et al.* (*Annalen*, 1924, **438**, 169; 1925, **445**, 35), is

$$\left[C_6H_5.CH_2\text{–}S\text{–}C\begin{matrix}NH_2\\NH_2\end{matrix}\right]^+ Cl^-$$

It is prepared as follows:—A mixture of 126 g. of benzyl chloride, 76 g. of thiourea and 200 ml. of alcohol is gently heated beneath a reflux condenser for 30 minutes.

The crude product, which forms a crystalline magma when cold, is purified by re-crystallisation from alcohol or from a mixture of equal parts of hydrochloric acid and water. It melts at 172 to 174° C., but exhibits dimorphism, the other variety melting at 146 to 148° C. The lower-melting variety is converted into that of higher m.p. by re-crystallisation from alcohol after seeding the solution with a few crystals of the higher-melting variety. The reagent is used according to the following procedure:—A concentrated solution of the sodium or potassium salt of the organic acid in water or aqueous alcohol is added rapidly to a slight excess of a 15 per cent. solution of the reagent in hot alcohol. On cooling the solution the S-benzyl thiuronium salt of the organic acid usually crystallises in a state of high purity and, if necessary, may be re-crystallised from alcohol. In a few instances, especially with the aliphatic hydroxy-acids, concentration of the solution is necessary before crystallisation takes place. With the weaker organic acids hydrolysis of the salt may occur, but this can be avoided by the use of non-aqueous solvents; with stronger acids, such as the sulphonic acids, an aqueous medium is to be preferred. The advantages of the method are the ease of preparation of the reagent, the rapidity of the operation, the ease of recovery of the acids, and the well-defined physical properties of the salts. The disadvantages are the restricted range of the melting-points of the salts of some of the fatty acids (*e.g.* propionic 148° C., *n*-butyric 146° C., *iso*-butyric 143° C., lauric 141° C., palmitic 141° C., stearic 143° C.) (but this difficulty can be overcome by using the method of mixed melting-points), and hydrolysis of the salts by careless manipulation and subsequent decomposition of the free base into benzyl mercaptan. The corrected melting-points of the salts of 39 organic acids are given. A. O. J.

Leaf Oils of Washington Conifers. C. Schwartz, Jr. (*Amer. J. Pharm.*, 1936, **108**, 197–200.)—*Abies lasiocarpa* (Hook), the "alpine fir," which is found in altitudes above 5000 ft., varies in height from 3 to 90 ft. The leaves and twigs (collected in September, 1933) yielded 0·78 per cent. of a volatile, pale yellow, very aromatic oil, readily soluble in 95 per cent. alcohol. The general constants of the oil were determined, and the following constituents were found to be present in the percentages stated:—*l*-β-pinene, 26·56; *l*-β-phellandrene, 24·0; esters (chiefly bornyl acetate), 16·45; free borneol, 7·76; *l*-camphene, 5·11; high-boiling residue, 4·44; *l*-α-pinene, 4·11; salicyclic acid (presumably as ester), 0·5; and traces of free acids. Sesquiterpenes, if present, occur only in small amounts.
D. G. H.

Some Characteristics of Wood Lignins. E. E. Harris. (*J. Amer. Chem. Soc.*, 1936, **58**, 894–896.)—The differences previously reported (Harris, Sherrard and Mitchell, *J. Amer. Chem. Soc.*, 1934, **56**, 889) between maple and spruce lignins prepared by the sulphuric acid method (Sherrard and Harris, *Ind. Eng. Chem.*, 1932, **24**, 103) have been found to exist between the lignins of other hardwoods (aspen, white oak, beech and basswood) and softwoods (spruce, slash pine and eastern hemlock). In the preparation of lignin from oak the wood was first extracted with alcohol (Ritter and Barbour, *Ind. Eng. Chem.*, 1935, **7**, 238) by which some substances not soluble in alcohol-benzene mixtures were removed.

Lignins isolated by extraction with methyl alcohol acidified with hydrochloric acid (Friedrich, *Z. physiol. Chem.*, 1928, **176**, 127; Brauns and Hibbert, *J. Amer. Chem. Soc.*, 1933, **55**, 4720) were also studied. Samples of lignin obtained by the two methods were methylated and chlorinated. Moist lignin was methylated by treatment with dimethyl sulphate in the presence of sodium hydroxide. Dried lignin was chlorinated by treatment with dry chlorine in dry carbon tetrachloride until it reached the second or light coloured stage. The chlorolignin showed about 20 per cent. increase in weight. With the exception of a higher yield of lignin from oak, a similarity exists between the hardwood lignins and another similarity exists between the softwood lignins. Except for the methoxyl-content of the fully methylated lignin and the chlorine-content of the fully chlorinated lignin, there are distinct differences between the hardwood and softwood lignins. The lignins isolated by the sulphuric acid method were partly soluble in alcohol or acetone—about 12 per cent. of hardwood lignin and 2 per cent. of softwood lignin. These soluble fractions had the same methoxyl-content, could be methylated to the same percentage of methoxyl and gave chlorolignins with the same percentages of methoxyl and chlorine as the corresponding insoluble fractions from which they were separated. The yield of lignin when the methyl alcohol method was used was low, *viz.* 30 per cent. of the lignin in hardwood and 15 per cent. of the lignin in softwood. To determine the effect of a higher reaction temperature higher-boiling solvents, *viz.* dioxane and cellosolve were used. Methyl cellosolve gave a yield of 90 per cent. of the total lignin in maple wood and dioxane 50 per cent. of the total. The methoxyl-content is higher in lignins prepared by the methyl alcohol and the methyl cellosolve methods. Brauns and Hibbert (*loc. cit.*) conclude that this increase is due to methylation of one of the hydroxyl groups during isolation. No increase in alkoxyl-content was found when dioxane was used. These products are easily methylated to give the same methoxyl-content as methylated lignin prepared by the sulphuric acid method. Methyl alcohol, methyl cellosolve and dioxane must also act as demethylating agents, because the lignin left in the wood after treatment contained less methoxyl. Demethylation has been observed by other workers (Heuser and Schmitt, *Cellulosechemie*, 1920, **1**, 49; 1921, **2**, 81), but under more drastic conditions and without simultaneous methylation. During chlorination both hardwood and softwood lignins lose methoxyl, and on the assumption that fully methylated lignin contains ten methoxyl groups, the loss is equivalent to two groups. After correcting for this loss the weight of chlorine introduced into lignin during chlorination is the same as the total increase in weight of the chlorolignin. The chlorine therefore acts by addition or substitution rather than by oxidation. Harris, Sherrard and Mitchell (*loc. cit.*) assigned ten methoxyl groups to the fully methylated compound containing 32 per cent. of lignin. Hardwood lignins isolated by the sulphuric acid method are shown to contain six methoxyl groups, and softwood lignins five methoxyl groups, and both varieties, when fully methylated, contain ten groups. The substances isolated by the methyl alcohol method are shown to be mixtures of lignin derivatives containing different numbers of methoxyl groups. A. O. J.

Inorganic

New Organic Reagent for Metals, particularly for Silver. S. E. Sheppard and H. R. Brigham. (*J. Amer. Chem. Soc.*, 1936, **58**, 1046–1049.)—From empirical and group analyses the new reagent is shown to be 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine. Glycine ethyl ester hydrochloride is treated with dry silver oxide in anhydrous ether, the ethereal solution of the free ester is dried over anhydrous sodium sulphate and, after evaporation of the greater part of the solvent, the residue is treated with absolute alcohol and excess of carbon disulphide beneath a reflux condenser. The di-ethylamino-acetate-dithiocarbamate is isolated and treated in the same manner with anhydrous alcohol and more carbon disulphide, a further excess of which appears to be necessary for the formation of the orange body. Hydrogen sulphide is eliminated at this stage. The crystals obtained are purified by re-crystallisation from ethylene chlorhydrin. The melting-point (uncorr.) is 276 to 280° C., and the approximate molecular weight, determined by depression of the freezing-point of thymol, is 200. The substance is very soluble in aniline, phenol, thymol, and hot ethylene chlorhydrin; soluble in hot acetophenone; slightly soluble in acetone, acetic acid, hot benzene, butyl alcohol, chloroform, ethyl acetate and heptane; insoluble in carbon tetrachloride, ligroin and water. It does not re-crystallise from solutions in acetophenone, benzene, butyl alcohol and chloroform. The compound gives precipitates of different colours with various metallic ions in neutral solution, but in acid solution only silver ions react to form a coloured insoluble compound. The colours given by approximately 0·01 g. of the metallic ion in 10 ml. of a neutral solution with 0·5 ml. of 0·03 per cent. solution of the reagent in acetone are as follows:—silver, purple; cadmium and ammonium, red; copper, red-blue; iron, yellow; lead, blue; zinc, pink; manganese and tin, white precipitates. In acid solution under the same conditions silver gives a purple solution, and the other metals give yellow solutions, with the exception of manganese and tin, which give white precipitates. The sensitivity was compared with that of Feigl's reagent (*Z. anal. Chem.*, 1928, **74**, 380; Abst., ANALYST, 1928, **53**, 615) by the procedure of Kolthoff (*J. Amer. Chem. Soc.*, 1930, **52**, 2222). To 10 ml. of silver solutions of varying concentrations 0·5 ml. of 4 *N* nitric acid was added, followed by 0·3 ml. of 0·03 per cent. solution of the reagent in acetone. Under these conditions the reagent detects 1 part of silver ion per million, whereas Feigl's reagent detects 1 part in five million. This is due to the fact that the blank solution of the new reagent is yellow, whilst the blank solution of Feigl's reagent is colourless. The sensitivity is increased by reducing to one drop the amount of 4 *N* nitric acid added. One part of silver ion per five million is then detectable. It is suggested, from analogy with Feigl's reagent, that the sensitivity of the compound as a reagent for silver ions would be increased if it were condensed with substituted benzaldehydes. A. O. J.

Separation of Iron from Copper and from Nickel. P. Spacu. (*Bull. Soc. Chim.*, 1936, **3**, 1061–1063.)—(i) *From copper.*—The hot neutral or faintly acid solution (100 to 150 ml.) containing the iron as ferric salt, is treated with pyridine,

drop by drop, until the iron is precipitated and the solution is blue. The precipitate is collected, washed with hot water, dissolved in hydrochloric acid, re-precipitated as before, ignited, and weighed as Fe_2O_3. The filtrate is concentrated to 100 to 150 ml., and treated in the cold with pyridine until deep blue, then with solid potassium thiocyanate. The green precipitate is ignited to copper oxide (*cf.* ANALYST, 1927, **52**, 494).

(ii) *From nickel.*—The separation is effected as described above. The nickel in the filtrate is precipitated as the complex $NiPy_4(SCN)_2$, and weighed as such (*ibid.*, 1927, 660). W. R. S.

Determination of Tellurium in Steel. E. Deiss and H. Leysaht. (*Z. anal. Chem.*, 1936, **105**, 323–325.)—The drillings (5 or 10 g.) are dissolved in nitric acid (1 : 1), the solution is evaporated to dryness, and the residue is strongly heated until nitric fumes are expelled. The residue is dissolved in strong hydrochloric acid, the solution is evaporated, and the residue is heated to 135° C. to render silica insoluble. The residue is again treated with hydrochloric acid, and the silica is filtered off and washed with acid. The filtrate is concentrated to 100 ml., and treated with sulphur dioxide and a crystal of potassium iodide. Tellurium is precipitated, and flocculates on continued treatment with the gas. The precipitate is collected in a porous porcelain crucible, washed with dilute hydrochloric acid, water, alcohol, and ether, dried for a short time at 105° C., and weighed. Rothe's ether extraction method cannot be used for the determination of tellurium, as this element distributes itself between the aqueous and ethereal solutions. W. R. S.

Determination of Manganese in Silicate Rocks. O. Hackl. (*Z. anal. Chem.*, 1936, **105**, 81–95, 182–199.)—A lengthy description of the author's procedure for the determination of manganese together with the main constituents of the rock, with an account of the tests that led to the adoption of the procedure. The method given below has been perfected by several years' practical application. The chloride solution from 1 g. of sample, obtained by the usual carbonate fusion, etc., is treated with a slight excess of ammonia and with hydrogen peroxide, and the precipitate formed is filtered off and partially washed. The precipitate and filter are returned to the beaker and well moistened in the cold with dilute nitric acid (1 : 1). When the precipitate has completely dissolved, as shown by the disappearance of the ferric hydroxide colour, hot water is added, and a second precipitation is carried out with ammonia and hydrogen peroxide, in presence of the pulped filter-paper. The precipitate and pulp are collected and washed with hot water containing ammonium nitrate until free from chlorine. The combined filtrates and washings are concentrated in a platinum dish to 150 or 100 ml., with occasional addition of 1 to 2 drops of ammonia for the precipitation of traces of alumina. If a precipitate is obtained at this point, it is collected and added to the bulk of the ammonia precipitate, which is ignited and weighed in the platinum crucible containing the weighed hydrofluoric acid residue from the silica determination. The weighed precipitate is fused in the same crucible with 8 g. of mixed sodium and potassium pyrosulphates (equal parts) for some hours. The acidity of the melt may be controlled by the fusion loss, which should be 0·7 to 0·8 g. The melt is dissolved

in warm water (100 to 120 ml.) and 10 ml. of sulphuric acid (1 : 1), the insoluble residue, if at all considerable, being collected, again fused with a maximum of 1 g. of pyrosulphate, and the solution added to the bulk.

The solution of the melt is treated with hydrogen sulphide, the platinum precipitate is filtered off, and the filtrate is concentrated to about 70 ml. and treated with 10 ml. of 2 per cent. silver sulphate solution. Any turbidity due to silver chloride is removed by agitation with filter-pulp and filtration, the washings being reserved and added to the filtrate after the manganese determination. Potassium (not ammonium) persulphate (0·5 to 1 g.) is added; the solution is diluted to about 90 ml. and heated with a small flame, the tip of which should not touch the bottom of the beaker (avoidance of local overheating and consequent partial peroxidation of titania). A thermometer is used as a stirrer, heating is arrested at 75° to 80° C., and after 3 to 4 minutes the beaker is quickly cooled. The liquid is transferred to a graduated 100-ml. flask, and the permanganate is determined in an aliquot part of the solution. The standard for the manganese determination is freshly prepared by dissolving 0·0045 g. of potassium permanganate in 100 ml. of water (10 ml. = 0·2 mg. MnO). If the rock is rich in iron, the permanganate is dissolved in water to which a saturated solution of ferric ammonium sulphate has been added until the tint of the standard solution matches that of the solution under investigation. The whole solution is next treated with hydrogen peroxide, which decomposes the permanganate and peroxidises the titania: this is determined colorimetrically. The solution is then evaporated to 100 ml., reduced with hydrogen sulphide in the cold for half-an-hour, and again for 15 minutes while being heated; if necessary, it may be filtered and again treated with hydrogen sulphide for a short time. The hydrogen sulphide is expelled by half-an-hour's boiling in a current of carbon dioxide, and the iron is titrated with permanganate after dilution. The author does not attach much weight to Lundell and Knowles's criticism of the reduction of iron by hydrogen sulphide (*J. Amer. Chem. Soc.*, **1921**, **43**, 1560) when performed as prescribed above. W. R. S.

Simultaneous Volumetric Determination of Oxalate and Hydrogen Peroxide. A. Simon and T. Reetz. (*Z. anal. Chem.*, 1936, **105**, 321–323.)—The method previously described (ANALYST, 1936, 356) may be simplified by omitting the addition of calcium nitrate solution in the treatment of the second portion. This is made alkaline with sodium hydroxide, boiled for 5 minutes after addition of 1 ml. of 0·1 *N* ferric chloride solution or 1 ml. of 0·001 *N* permanganate, then acidified and titrated as before. W. R. S.

Microchemical

Electrometric Determination of Bromine in the presence of large amounts of Chlorine. G. E. Vladimirov and J. A. Epstein. (*Mikrochem.*, 1935, **18**, 58–65.)—The electrometric method used gives excellent results with mixtures of similar amounts of chlorine and bromine salts. In biological material, however, the chlorine is greatly in excess of the bromine, and it is necessary to reduce this excess by extraction with acetone, in which bromides are soluble.

Determination of bromine in mixtures of the pure salts.—The solution of sodium chloride and bromide is evaporated to dryness in a centrifuge tube. The dry residue is dissolved in 0·2 to 0·35 ml. of water, and 5 ml. of anhydrous acetone are added. Most of the chloride is deposited and can be separated by centrifuging. The supernatant liquid is decanted, and the residue is mixed with a drop of distilled water and 2 ml. of acetone and treated as before. The combined acetone extracts are evaporated to dryness, and the residue is dissolved in 3 to 5 ml. of a 5 per cent. solution of barium nitrate, which increases the accuracy of the determination as it prevents the adsorption on the silver halide of the halogen ions in solution. A thick ring of silver wire forms the electrode, and a supersaturated solution of sodium nitrate is used as a bridge in a bent tube stoppered with cotton wool; this connects the test solution with the standard electrode, which is a quinhydrone electrode with 0·01 *N* hydrochloric acid. The potential difference is determined by the usual compensation method, with the use of a mirror galvanometer. For the titration 0·01 *N* silver nitrate is used, and the end-point is obvious, owing to the large jump in the potential. The zone of potential change fluctuates slightly with the dilution and amount of chloride present, but the titration error is not more than 0·02 ml., which implies an error of 1 to 6 per cent. The least quantity of bromine that can conveniently be determined by this method is 0·24 mg. in a solution in which 170 times the amount of chloride was originally present. *Application to blood and tissues.*—The silver halides are precipitated and the organic matter is oxidised by heating with conc. nitric acid and hydrogen peroxide, after which the silver halide is converted into the sodium salt by reduction with sodium amalgam, the excess of alkali is neutralised with sulphuric acid, the sodium sulphate formed is removed with alcohol, the filtrate evaporated to dryness on the water-bath, and the residue is treated with acetone as described. *Detail.*—Two ml. of blood serum are heated on the water-bath with 7 ml. of *N* silver nitrate solution in conc. nitric acid, until the supernatant liquid is clear. It is sucked off, a few ml. of conc. nitric acid and a few drops of silver nitrate solution are added, the mixture is heated on the water-bath, and perhydrol is cautiously added until the precipitate sinks to the bottom of the tube as a powder. The supernatant liquid is sucked off, and the precipitate is washed twice with water and dissolved in 2 ml. of water. Any iodine present is volatilised in this treatment, but there is no loss of bromine. About 0·5 to 2·0 g. of freshly-prepared sodium amalgam are used for the reduction, which takes about 15 minutes, after which the alkali is neutralised to phenolphthalein with *N*-sulphuric acid, and the mixture is evaporated 1 to 2 ml. and treated with about 5 times the volume of alcohol to remove the sodium sulphate. The error with blood serum is of the order of 4 per cent. J. W. M.

Berberine as a Microchemical Reagent. C. Van Zijp. (*Pharm. Weekblad*, 1936, **73**, 764–767.)—If a large drop of water is mixed with a drop of 4 *N* hydrochloric acid and a crystal of berberine sulphate is added, broad dichroic prisms are produced on scratching; they are *d*-rotatory and strongly anisotropic, n_α being in the long direction of the crystal. This method is preferable to the similar reaction involving the use of nitric acid. If a drop of a solution containing

a little berberine compound is held over vapours of ammonia and then evaporated in daylight, few, if any, crystals result, but if this procedure is carried out in the presence of the following compounds, definite reactions are obtained which are useful as an aid to identification:—Uric acid gives fine needles without the necessity of evaporation; they frequently form radiating groups, and are dichroic and brown in colour when viewed by transmitted light, n_a being in the long direction of the crystal. Theocine produces no immediate crystallisation, but on evaporation radiating needles result which can be distinguished from those produced by uric acid by examination under crossed Nicols. Luminal and its sodium compound produce groups of radiating yellow needles, and atophan gives similar results except that the crystals are white. The crystals from rutonal and dial are similar in shape and distribution, but are shorter, coarser and not dichroic, whilst the former differ from the latter in that the groups it forms are less compact. Very short wide crystals forming small balls with a granular appearance are obtained from propanal, and veronal deposits a relatively small number of single yellow needles which sometimes form star-shaped groups and do not increase in number on evaporation. After a long period evipan gives an indistinct reaction, long yellow radiating needles being formed. Salicylic acid, sodium salicylate and aspirin produce *d*-rotatory needles, saccharin forms shorter needles, and with fumaric acid strongly anisotropic needles in tufts and radiating groups are obtained. With meconic acid the only crystals normally visible in the absence of ammonia are the short needles of berberine sulphate itself, but if the reaction is applied as described above, these are seen to be overgrown with tufts of finer needles. Theobromine, cystine, leucine, codeine, urea and anisic, cinnamic and succinic acids give negative results, whilst tyrosine, although it dissolves in the drop, reacts only with difficulty, forming radiating groups of fine crystals (*cf. id.*, 1930, **67**, 198).

J. G.

Micro-reaction of Caffeine with Iodine in Potassium Iodide Solution. C. Van Zijp. (*Pharm. Weekblad*, 1936, **73**, 767–768.)—A small quantity of the specimen is stirred into a fairly large drop of water, and a small drop of a solution containing 1 g. of iodine and 2 g. of potassium iodide in 9 g. of water is added. The mixture is allowed to evaporate at 40° to 50° C., when dark brown drops form, which, when allowed to cool and scratched, deposit well-formed, regular, red-brown dichroic, lozenge-shaped crystals, with an acute angle of 86° (which gives them at first sight the appearance of squares). If sodium iodide is substituted for potassium iodide, brown drops are obtained, but these do not crystallise. J. G.

Nephelometric Determination of small Amounts of Nicotine. R. Hofmann. (*Mikrochem.*, 1935, **18**, 24–30.)—Amounts of nicotine of the order of 0·15–0·23 mg. may be determined by the use of a micro-nephelometer for 5 ml. of solution (15 ml. for 3 measurements), with an error of 2 per cent. The standard solution adopted was a 0·03665 per cent. solution of pure nicotine, containing 5 per cent. of hydrochloric acid, and the reagent a solution of silico-molybdic acid made up as previously described (*Biochem. Z.*, 1933, **260**, 26). The acid-content of the test and comparison solutions should be similar, preferably

0·5 per cent. The first determination is approximate, to ascertain the most suitable dilution to use. The best concentration of nicotine for turbidity measurement is of the order of 0·0015 per cent. It was found that the eye is less tired when only one reading of the nephelometer is taken for each light intensity; three light intensities from 10, 15 and 20 mm. light openings were used. *Detail.*—Tobacco (*e.g.* 0·5 to 1 g.) is distilled in steam, and 100 ml. of distillate are collected. The correct dilution for measurement is found most rapidly by taking 5 reagent tubes, placing 5 ml. of 0·5 per cent. hydrochloric acid in each, and then 5 ml. of the distillate in the first tube, 5 ml. of this mixture in the second tube, and so on, and finally 1 ml. of the silicomolybdate reagent in each tube. The dilution which gives a turbidity slightly greater than that of the standard is selected and 100 ml. are prepared by taking the correct volume of distillate (usually about 25 ml.), neutralising to methyl red with 0·1 *N* hydrochloric acid, adding 10 ml. of 5 per cent. hydrochloric acid and diluting the mixture to 100 ml. This solution is placed in a burette, and varying amounts (*e.g.* 4 to 5 ml.) are run into dry reagent tubes and diluted to 10 ml. with 0·5 per cent. hydrochloric acid from a burette. Two ml. of reagent are then added, and the turbidity is compared with that of the standard. Results compared with those given by the gravimetric analysis showed rather large errors—up to 10 per cent. The errors were found to be due to the difference in particle size between the precipitate formed from pure nicotine, and that from tobacco distillate (nicotine 0·1137μ, distillate 0·0963μ); hence a standard solution made from tobacco distillate of known nicotine-content was used for comparison, and in this way the error was reduced to 2 per cent.

J. W. M.

Copper Catalysis of the Oxidation of Thiol Acids as a Basis for the Micro-determination of Copper. J. Bjerrum. (*J. Biol. Chem.*, 1936, **114**, 357–359.)—With thioglycollic, thiolactic and thiomaleic acids oxidation by air in *N*/10 to *N* hydrochloric acid solutions occurs in the presence of traces of copper. The thioglycollic and thiolactic acids are oxidised to disulphide acids, and the unoxidised acid can be titrated iodimetrically. For a given thiol acid concentration the rate of oxidation is proportional to the copper concentration, up to a critical (very small) copper concentration. The specificity of the reaction was examined for glycollic acid. With mercuric ions, precipitation occurred in 0·0003 *M* solution; with ferric ions 1 equivalent of thiol acid was oxidised; neither had any other effect. Fluoride increased the copper catalysis and thiocyanate retarded it. Experiments on copper catalysis were not reproducible with an accuracy higher than 10 to 15 per cent., and it is not possible to work out a better method than Warburg's cysteine oxidation. For small amounts of copper, however, iodimetric estimation is possible. Vacuum-distilled thioglycollic acid, kept as approximately *M*/10 in *N* HCl, was diluted to *N*/4 hydrochloric acid, and 4-ml. samples were shaken mechanically for 18 hours at 25° C. with human blood serum (0·25 and 0·5 ml.), with varying amounts of copper sulphate, and with mixtures of both, in conical flasks, small, but very much larger than the volume of liquid. The thiol acid concentration was 0·0245 *M*. The serum examined contained a little more than 1 mg. per litre. This was in agreement with the results of the cysteine oxidation.

Similarly, the copper-content of cow's milk was found to be 0·05 to 0·07 mg. per l., agreeing with recent determinations (McFarlane, *Biochem. J.*, 1932, **26**, 1030; Abst., ANALYST, 1932, **57**, 803). E. B. D.

Centrifuge with Removable Tip for Gravimetric Work. S. D. Elek. (*Mikrochem.*, 1936, **19**, 129–131.)—This is an improvement on the Friedrich centrifuge tube with removable tip, as the use of Krønig's cement for the ground-glass joint is avoided by binding the two parts of the tube in place at the ground joint, using a split metal ring with thread outside on the upper portion of the tube, and screwing this in place by means of a slightly tapered ring with thread inside. The cap of the centrifuge tube fits inside the metal ring, but is separated from it by a rubber ring, so that the fit is firm and there is no danger of the glass cracking. The tip of the centrifuge tube is much more easily cleaned for weighing than that of the Friedrich tube. J. W. M.

Physical Methods, Apparatus, etc.

Radiography of Cloth. H. F. Sherwood. (*J. Text. Inst.*, 1936, **27**, 162–170T.)—The only practicable procedure with structures which are too fine to be seen with the naked eye is to radiograph the specimen on a fine-grained plate, and to examine the photograph with a lens or to enlarge it (*e.g.* up to 75 diameters; *cf.* Fricke, *Radiography and Clin. Phot.*, 1932, **8**, 12; Sherwood, *id.*, 1934, **10**, 10). X-rays of long wave-lengths ("soft" X-rays or "Grenz"-rays) should be used, as they have low penetrating powers, but a special type of X-ray tube fitted with an extremely thin window to facilitate the escape of the rays is required, and the voltages concerned (12 to 15 kilovolts) are quite low compared with those necessary for medical work or for the radiography of heavier materials. In the Westinghouse instrument described, the specimen is mounted close to the plate with a No. 87 Wratten (infra-red) filter between, to afford protection from ordinary light, but at the same time to allow the passage of the X-rays. This filter has been found to be sufficiently homogeneous in structure, but thin black paper is unsuitable because its structure is recorded by the X-rays. The remainder of the apparatus consists of an X-ray tube in a metal protecting shield, with a focal spot to direct the rays through a window (12 to 18 microns thick) on to the specimen; a filament is inserted at right angles to the path of the rays. A special film-holder, which may be loaded in daylight, is also described; it is arranged so that 6 areas of size $2\frac{1}{4} \times 3\frac{1}{4}$ inches (or twice the number having half this area) may be exposed in succession, the film being protected from exposure to light by an ultra-violet filter. A special film having a fine grain and a high sensitiveness to Grenz-rays is used, and positive prints may be made from it in which the lightest areas represent the least degree of absorption of X-rays by the specimen. Exposure-periods range from 5 to 120 seconds; tube-voltages, 4 to 12 Kv.P., according to the thickness of the specimen; and the distances between the anode and the film, 5 to 12 inches. Radiographs of cloth bear some resemblance to the visual appearance of the weave, although the effects produced by the entire thickness in absorbing X-rays are shown, instead of only the surface characteristics. Tightly-twisted strands absorb X-rays more strongly than a like strand loosely twisted.

Silk weighted with lead salts (*e.g.* 40 per cent. by weight) absorbs more than a silk weighted with 28 per cent. of tin salts, and this in turn is more absorbent than pure silk. A strongly-absorbent printed stripe parallel to the warp and across the filling indicates the presence of a heavy element. X-rays show less absorption for warp streaks in taffeta, indicating that these have been stretched during the weaving process; this suggests a method of distinguishing between streaks caused by stretched warp-ends and those due to uneven absorption of dyestuffs. Most dyestuffs contain elements of low atomic weights which have only a slight absorption for X-rays, and dyestuffs containing elements of higher atomic weights are revealed by an increased opacity to X-rays. The above-mentioned examples are illustrated by enlarged radiographs. The method may also be applied to the examination of insects, plant structures, paper, etc.

J. G.

Fluorescence Phenomena. I. Fluorescent Minerals. II. Chelidonine, a Fluorescent Principle. III. Neville-Winther Acid as a Fluorescent Indicator. M. Déribéré. (*Ann. Chim. Anal.*, 1936, **18**, 117–120.)—I. The following fluorescence effects are recorded:—autunite-uranite, uranocircite or uranium nitrate, an intense green, characteristic of uranium salts and enabling these minerals to be distinguished from the radium minerals; blende, bright orange; willemite-troostite, brilliant green*; chalcolite, intense green (attributable to the uranium constituents rather than to the copper); celestine, beige; scapolite, bright orange-yellow; fluorite, deep violet; calcite or gypsum, dull red; aragonite, bright red; rock salt, yellow (owing to inclusions of petrol in the cubic crystals); sodalite, orange; sylvinite, pale red; zircon (commercial quality), orange-yellow. The method is helpful as an aid to identification and classification control, but it should be used as a supplement to other methods, as small traces of certain impurities have considerable influence on the appearance of the fluorescence.

II. Aqueous or alcoholic extracts (0·05 to 2 per cent.) of the sap of the celandine (*Chelidonium majus*) are yellow, and have a strong golden-yellow fluorescence which decreases in intensity on further dilution or when the *p*H exceeds 12, but is unaffected by reduction of the *p*H value. Cloth, wood, oils or paper coloured with these extracts also show the fluorescence.

III. If a saturated solution of α-naphthol 1 : 4 sulphonic acid (Neville-Winther acid) is diluted with water until the yellow colour disappears and the liquid appears limpid (*i.e.* approximately 1 drop in 10 ml.), the resulting liquid serves as a fluorescent acid-alkali indicator, a blue fluorescence being visible at *p*H 6·5 or over and disappearing at *p*H 6·0 or below. Addition of salts of the alkali or alkaline-earth metals, or of formaldehyde, is without effect on the change, but the concentration of the indicator has a considerable influence on the intensity of the fluorescence, which is zero for saturated solutions and a maximum under the conditions described above (*cf. id.*, 1936, **18**, 37). J. G.

* Abstractor's Note.—The fluorescence of willemite varies considerably according to the place of origin.

Reviews

PERFUMES, COSMETICS AND SOAPS, WITH SPECIAL REFERENCE TO SYNTHETICS. By W. A. POUCHER, Ph.C. Fourth edition. Vol. I. Pp. xx + 439, with 40 illustrations. London: Chapman & Hall. 1936. Price 25s. net.

Though only first published as one volume in 1923, this work has now reached its fourth edition, and so much new matter has been added in successive editions that it has now become necessary to divide it into three. In this latest edition, Vol. I remains a "dictionary of raw materials" used in the industry, and, of more than 100 new substances included for the first time, it is noteworthy, as reflecting the increasing demand for cosmetics, that 32 are "cosmetic constituents," in contradistinction to essential oils or synthetics. The practice adopted in the last edition, of giving the chemical formulae and physical constants for most of the synthetics, has been continued and extended; unfortunately, however, a few errors in these formulae have been overlooked in the revision of the proofs.

Most of the reviewer's criticisms of the earlier editions have now been met, but in the new matter cholesterol (cholesterin) is misspelt cholestrol (cholestrin), which may lead to confusion, and its formula is given wrongly as $C_{26}H_{44}O$ instead of $C_{27}H_{46}O$; the melting-point of cetyl alcohol is said to "range from 30° to 50°," whereas, if reasonably pure, it should melt at about 50°; and it is rather surprising, under arachis (groundnut) oil, to be referred to Katchung oil, a name rarely, if ever, met with, at any rate in this country. The glyceride present in Japan wax and myrtle wax is of course palmitin, not palmatin. The statement that linaloe oil "has now practically disappeared from commerce" appears rather too sweeping, and among the very few omissions noted there is no reference to the Indian linaloe oil, now being produced from trees grown in India from seed obtained from Mexico, nor to massoia bark oil, which is an article of commerce.

One valuable feature of this work has always been the very large number of formulae for the reproduction of natural perfumes by mixtures of synthetics. Many of these formulae have now been revised and modified in view of recent developments in the variety of synthetics available, and of their improved quality.

The book is well illustrated, several of the plates being new, and is attractively bound in leatherette. It can be confidently recommended to the growing number of chemists who are interested in the production or examination of cosmetics and allied substances, and this edition should undoubtedly enhance the reputation of a work which has already established itself as a standard book of reference on the subject.

W. H. SIMMONS

LAUNDRY CHEMISTRY. By A. HARVEY. Second edition. Pp. vii + 118. London: The Technical Press, Ltd. 1935. Price 4s.

This manual, the first edition of which was reviewed in THE ANALYST (1927, p. 62), is intended for those engaged in laundry practice who desire to acquire the scientific principles underlying the use of the various substances employed in the laundering of textiles.

The present edition is on lines similar to those of the previous one, and, in addition, provides information on the newer materials adopted in laundry technique.

The subject-matter is treated in a clear and concise manner, but too much space is devoted to the manufacture of such substances as sodium perborate, sodium carbonate, chlorine, and the like—information for which the readers of the book will have little use.

It is claimed that this edition has been completely revised, but several minor defects remain, including either incomplete or incorrect equations on pages 31, 61, 64 and 82. "Copper hydrate" is referred to on p. 90, where the hydroxide is intended, and on looking up the concentration of sodium perborate for use as bleaching agent we are provided with the undefinable proportion of "1 oz. to a shirt machine." On p. 107, dilute hydrochloric acid is recommended for the removal of iron mould, but, in the reviewer's experience, the fabric usually disappears before the stain when this reagent is employed.

However, these defects detract little from the undoubted merits of the volume, which is a valuable contribution to one branch of industrial chemistry; but it may be suggested that more interest would be added to the tables on pages 2 and 18 if the foreign origins of the element symbols in the one, and the sources of the waters of which analyses are given in the other, had been provided.

T. J. WARD

MICROSCOPE SLIDE MAKING. By CHAS. E. HEATH, F.R.M.S. Pp. 77, with 18 illustrations. London: Marshall & Co., Ltd. Price 1s. 6d. net.

This small volume, which is intended for the use of those commencing the mounting of microscopic slides, provides practically all the non-specialised instruction required in the preparation of a wide variety of objects by several methods, including microtomy and grinding.

A brief introduction is followed by a chapter giving descriptions and uses of the necessary tools and materials, some of which may be adapted from domestic appliances; the remainder of the text is devoted to the methods of mounting.

The information throughout is sound, and evidently based upon wide experience, and the reviewer concurs with the author in his claim that the mounting of microscopic objects is excellent training in accuracy of observation and dexterity, whilst the cost of the necessary appliances is relatively small. The text is unusually free from errors, but a minor omission occurs on p. 20, and a little confusion between singular and plural on p. 22, and the use of the word "density" instead of refractive index on p. 28 is hardly correct. In spite of its low price the volume is a reliable and valuable guide, which will prove serviceable over a long period, and it is therefore to be regretted that the cover is not of a more durable nature than the thick paper provided.

T. J. WARD

A BRIEF COURSE IN QUALITATIVE CHEMICAL ANALYSIS. By LOUIS J. CURTMAN. Pp. viii + 245. New York: The Macmillan Company; London: Macmillan & Co. 1936. Price 10s.

Prof. Curtman has incorporated in this book the knowledge and experience gained during the teaching and practice of elementary analysis for many years. The book is written for the student-novice, and a tendency to "spoon-feeding" may be apparent, but the insistent emphasis laid on the need for a full realisation of the

theoretical aspect of the reactions involved, and upon the necessity for scrupulous care and attention to detail in practical manipulation, is to be commended.

The book is divided into four sections. The first (pp. 71) is a concise but adequate exposition of the laws of chemical equilibrium, more especially those relating to ionisation, solubility product, complex ion formation and oxidation-reduction. The reactions of metal ions and the acids are described in the second part (pp. 63), the actual scheme of analysis being set forth in the third part (pp. 66). A fourth short section (pp. 14) is devoted to the solution of problems related to analysis, which are used throughout the book as a means of emphasising theoretical considerations. Tables of logarithms, solubilities and solubility products are provided.

A few exceptions to the standard well-tried methods generally employed have been incorporated to give greater accuracy or ease of manipulation, and provision is made for the rough estimation of each element during its detection. The single test for nitrates appears somewhat inadequate; the lack of provision for dealing with "insolubles" is to be regretted.

The book is well planned and has been written and printed with a clarity well suited to its purpose. L. A. WARREN

DIE QUANTITATIVE ORGANISCHE MIKROANALYSE. By FRITZ PREGL. Fourth Edition. Re-written and enlarged by HUBERT ROTH. Pp. xiii+328, with 72 diagrams. Berlin: Springer. 1935. Price (bound) RM.26.

This is the first edition of Pregl's book published since his death in 1928, and as methods of micro-analysis are developing rapidly, a new edition was much needed. The book has been completely re-written by Dr. Roth, a considerable amount of new matter has been introduced, and a number of diagrams have been redrawn, and twenty more added. The arrangement follows that of Pregl, and the style is in the same tradition, with great emphasis on detail, so important in accurate small-scale work. Unfortunately, the index is not sufficiently detailed, so that a considerable familiarity with the methods and with Pregl's book is necessary in order to find a particular item of information. New methods take their places under the determination of elements, specific groups or physical constants.

The chapter on micro-balances is much improved, and the two best models of Kuhlmann and Bunge balances are described in detail. A point of interest is that the author has had two models of Bunge balance in daily use for five years, and has not observed the slightest deterioration.

The description of the Pregl combustion method is little changed; anhydrone (magnesium perchlorate) has been found to give good results for the absorption of water, and is much cleaner to handle than phosphorus pentoxide, which is generally used in this country. New matter includes the Zacherl and Krainick wet method for the determination of chlorine and bromine, the titration of amino-acids, Van Slyke's method for the determination of amino groups, the author's methods for the determination of active hydrogen, C-methyl groups and *iso*propylide groups, and a method for the determination of the number of double bonds.

The section on the determination of physical constants includes the useful method of determining the melting-point of crystals under the microscope and

Schleiermacher's micro boiling-point method; Emich's similar, but rather simpler, method is not mentioned. The measurement of absorption spectra and the determination of molecular refraction and specific rotation are also described.

The new edition is thus a complete handbook of organic micro-methods, and covers all the quantitative methods in general use, so that the organic chemist can now, if he wishes, scrap all his apparatus for obsolete macro-quantitative methods and adopt micro-methods throughout.

The author is to be congratulated in having brought together so much new material without altering the character of Pregl's book. It is to be hoped that a translation will soon be available. JANET W. MATTHEWS

Publications Received

TABLES OF PHYSICAL AND CHEMICAL CONSTANTS AND SOME MATHEMATICAL FUNCTIONS. Eighth Edition. By C. W. C. KAYE and T. H. LABY. Pp. v + 162. London: Longmans, Green & Co. Price 14s. net.

ELEMENTARY QUANTITATIVE ANALYSIS. THEORY AND PRACTICE. By H. H. WILLARD and N. H. FURMAN. Second Edition. Pp. x + 436. London: Macmillan & Co. Price 14s. net.

PERFUMES, COSMETICS AND SOAPS. By W. A. POUCHER. Vol. III. Being a Treatise on Modern Cosmetics. Fifth Edition. Pp. xi + 228. London: Chapman & Hall. Price 21s. net.

THE SCIENTIST IN ACTION. By W. H. GEORGE. Pp. 355. London: Williams & Norgate. Price 10s. 6d. net.

DIE FERMENTE UND IHRE WIRKUNGEN. Supplement: Lief. 3 und 4. By CARL OPPENHEIMER. Pp. 321–480 and 481–640. The Hague: W. Junk. Price 28s. each part.

HANDBUCH DER KAKAVERZEUGNISSE. By H. FINCKE. Pp. xiv + 568. Berlin: Julius Springer. Price (bound) RM.55.

PRACTICAL EVERYDAY CHEMISTRY. By H. BENNETT. Pp. 305. London: Spon. Price 10s. 6d. net.

A SHELLAC PATENT INDEX. By R. W. ALDIS. Pp. iv + 115. Indian Lac Research Institute, Nankum, India. Price Rs. 2/8.

RESEARCH ON THE LOW POTENCIES OF HOMOEOPATHY. By W. E. BOYD. London: Heinemann (Medical Books) Ltd.