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An Inquiry into Some Problems Connected with Milk.

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(*Read at the Meeting of the North of England Section, November* 30, 1929.)

The Rising of Fat in Milk.—Richmond (*Dairy Chemistry*, 1914, pp. 168–9) records the figures of three experiments which he conducted with a churn having a bottom tap, and one by "dipping" from the top milk in a pan, which show that within thirty minutes in two cases, and within fifteen minutes in the other two, rising of fat begins, but the milk he experimented with in the first test must have been six or even seven hours old, as the test was started at 11.25 a.m., and there is nothing to indicate the age of the milk in the others.

A series of experiments intended to imitate the practice of a farmer who used no cooler was therefore made; the yield of six cows, measuring about eight gallons, was "siled" direct into a ten-gallon churn, which was carried straight into the dairy, and one gallon was at once poured from the churn into a gallon measure, transferred to a two-gallon carrying can in which it was mixed with a plunger and a sample taken; this method of procedure was repeated at intervals of twenty minutes until the whole of the milk had been poured from the churn.

Nothing was done either to cool or stir the milk, and it received no agitation except that which was unavoidable in tilting the churn for pouring off the samples; the analysis of these samples is given in Tables I, II, III.

TABLE I.

Sample No.	Date.	Time.	Non-fatty solids. Per Cent.	Fat. Per Cent.
378	28/3/29	5.36 a.m.	8·93	3·47
379	,,	5·56 ,,	8·98	3·45
380	,,	6.16 ,,	9·02	3·42
381	,,	6.36 ,,	9·00	3·47
382	,,	6.56 ,,	8·89	3·87
383	,,	7.16 ,,	9·01	3·37
384	,,	7.36 ,,	8·91	3·37
385A	,,	7.56 ,,	8·84	3·45

Dairy temperature at 5.30 a.m., 50° F.

TABLE II.

Date:—24th July, 1929.

Time.	Non-fatty solids. Per Cent.	Fat. Per Cent.
5.15 a.m.	8·86	3·42
5.30 ,, ...	8·80	3·40
5.55 ,, ...	8.85	3.40
6.15 ,, ...	8·72	3·50
6.35 ,, ...	8·83	3·40
6.55 ,, ...	8·81	3·35
7.15 ,, ...	8·79	3·50
7.20 ,, ... (Last in churn.)	8·86	3·40

The last portion in the churn measured about one-and-a-half gallons.

Initial temperature at 5.15 a.m., 80·6° F.; final temperature at 7.20 a.m., 71·6° F.; dairy temperature, 66·2° F.

TABLE III.

Date:—26th September, 1929.

Time.	Non-fatty solids. Per Cent.	Fat. Per Cent.
5.35 a.m.	8·82	3·12
5.55 ,, ...	8·81	3·30
6.15 ,, ...	8·89	3·22
6.35 ,, ...	9·07	3·10
6.55 ,, ...	9·08	3·07
7.15 ,, ...	9·12	3·17
7.35 ,, ...	9·11	3·00
7.35 ,, ... (Last in churn.)	9·03	3·05

The last portion in the churn measured about two-and-a-half gallons.

Initial temperature at 5.35 a.m., 87·8° F.; final temperature at 7.35 a.m., 77·0° F.; dairy temperature, 54·5° F.

Further tests were made in which samples were taken from two lots of uncooled milk and from a cooled milk, but the time interval was lengthened; the figures found on analysis are included in Table IV.

TABLE IV.

Mar:	"A" Uncooled.	"B" Cooled.	"C" Uncooled.
Time:	6.5 a.m.	6.10 a.m.	6.0 a.m.
	Per Cent.	Per Cent.	Per Cent.
Non-fatty solids	9·18	8·84	9·20
Fat	3·10	3·27	3·15
Time:	7.5 a.m.	7.7 a.m.	7.10 a.m.
	Per Cent.	Per Cent.	Per Cent.
Non-fatty solids	9·30	8·81	9·10
Fat	3·10	3·93	3·47
Time:	8.50 a.m.	8.53 a.m.	8.58 a.m.
	Per Cent.	Per Cent.	Per Cent.
Non-fatty solids	9·22	8·39	8·85
Fat	3·47	6·90	4·62

Dairy temperature at 7 a.m., 56° F.

The temperature of the milk in each of the churns was taken at 7 a.m. and 9 a.m.:

	7 a.m.	9 a.m.
Churn "A," Uncooled	79° F.	76° F.
,, "B," Cooled	60° F.	60° F.
,, "C," Uncooled	78° F.	76° F.

To ascertain the effect of other methods of sampling, a test was carried out under the same conditions as before, excepting the actual withdrawal of the samples, which in this case were obtained by "dipping" from the top milk in the churn with a half-pint measure at twenty-minute intervals; the figures in Table V show the result of this experiment.

TABLE V.

Date:—24th July, 1929.

Time.	Non-fatty solids. Per Cent.	Fat. Per Cent.
6.02 a.m.	8·89	3·65
6.22 ,, ...	8·89	3·65
6.42 ,, ...	8·84	3·83
7.02 ,, ...	8·83	4·00
7.22 ,, ...	8·78	4·67
7.42 ,, ...	8·79	5·02

Next, a fifteen-gallon churn was fitted with three taps, the top tap at the ten-gallon mark, the middle tap at the five-gallon mark, and the bottom tap at a point one-and-three-quarter inches above the bottom of the churn.

Each tap was fitted on the inside with a glass tube of about a quarter inch internal diameter, and so arranged that the ends of the tubes were coincident with the perpendicular axis of the churn, so that samples would be drawn from the centre of the bulk of milk.

The milk of six cows was siled directly into this churn after milking, and samples were drawn simultaneously from the taps at twenty-minute intervals over a period of two hours; a final sample was taken from the top milk by "dipping." The results are given in Table VI. (About 6 ozs. was taken for each sample.)

TABLE VI.

Date:—24th July, 1929.

Time.	"Top tap." Non-fatty solids.	"Top tap." Fat.	"Middle tap." Non-fatty solids.	"Middle tap." Fat.	"Bottom tap." Non-fatty solids.	"Bottom tap." Fat.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
5.40 a.m. ..	8·97	3·20	9·07	3·17	9·02	3·22
6.00 ,, ..	8·97	3·25	9·00	3·22	8·99	3·20
6.20 ,, ..	9·01	3·20	9·04	3·17	9·00	3·07
6.40 ,, ..	8·97	3·25	9·04	3·07	8·94	3·00
7.00 ,, ..	8·80	3·32	9·09	2·87	9·07	2·60
7.20 ,, ..	9·03	3·02	9·13	2·72	9·22	2·30
7.40 ,, ..	9·03	2·87	9·18	2·60	9·15	2·25
7.40 a.m.—Last sample of series taken by dipping from top of churn					8·82	5·35

Three further experiments were made with the three-tap churn, but in these no glass tubes were used; in the first two tests of this series, after the churn had been filled to the twelve-gallon mark, its contents were stirred with the plunger before samples were taken at twenty-minute intervals from the taps, and also by "dipping" from the top milk; at the end of the period, in one test a sample was taken from the residue in the churn after plunging, by "dipping" from the top milk, while in the others samples were taken both from the top milk and from the bottom tap after the same treatment, but in the third test of this series fifteen gallons of milk were siled direct into the churn and the twenty-minute interval samples of a pint each were taken without any preliminary plunging, but the residue in the churn at the end of two hours and forty minutes was plunged and samples were drawn both from the top milk and from the bottom tap; Tables VII, VIII and IX embody the results of these experiments.

Table VII.

Date:—26th September, 1929.

Time. a.m.	"Top milk." Non-fatty solids. Per Cent.	Fat. Per Cent.	"Top tap." Non-fatty solidsl Per Cent.	Fat. Per Cent.	"Middle tap." Non-fatty solidsl Per Cent.	Fat. Per Cent.	"Bottom tap." Non-fatty solids. Per Cent.	Fat. Per Cent.
5.10	9·05	2·85	8·93	2·87	8·99	2·82	8·76	2·87
5.30	9·00	2·90	8·87	2·92	8·97	2·85	8·84	2·90
5.50	9·02	2·95	8·87	2·95	8·83	2·90	8·89	2·90
6.10	9·03	3·00	8·84	2·95	8·88	2·90	8·91	2·85
6.30	9·05	3·10	8·84	2·95	8·78	2·95	8·94	2·80
6.50	9·07	2·87	8·95	2·90	8·81	2·95	8·87	2·85
7.10	9·04	3·42	8·97	2·90	8·83	3·00	8·91	2·75
7.30	8·98	3·52	9·02	2·87	8·73	3·00	8·90	2·72
7.50	8·97	3·10	8·96	2·85	8·85	3·00	8·96	2·57
8.10	8·69	6·27	8·96	2·75	8·82	3·05	8·97	2·50

8.14—Last sample of series taken by dipping from top of churn after plunging .. .. 8·77 2·95

Table VIII.

Date:—12th November, 1929.

Time. a.m.	"Top milk." Non-fatty solids. Per Cent.	Fat. Per Cent.	"Top tap." Non-fatty solids. Per Cent.	Fat. Per Cent.	"Middle tap." Non-fatty solids. Per Cent.	Fat. Per Cent.	"Bottom tap." Non-fatty solids. Per Cent.	Fat. Per Cent.
5.5	9·01	3·25	9·04	3·25	9·16	3·20	9·01	3·20
5.25	9·05	3·45	9·10	3·25	9·15	3·25	9·01	3·20
5.45	9·01	3·50	9·18	3·25	9·13	3·20	9·07	3·25
6.5	9·06	3·30	9·00	3·30	9·18	3·20	9·04	3·20
6.25	9·03	3·45	9·04	3·30	8·96	3·20	8·94	3·32
6.45	9·08	3·45	9·08	3·30	9·01	3·20	9·02	3·25
7.5	9·16	3·60	9·02	3·40	9·02	3·20	9·03	3·15
7.25	9·09	4·05	8·94	3·40	8·94	3·20	8·94	3·15

7.32—Last samples of series taken from top by dipping and from bottom tap after plunging contents of churn:

Top milk: Non-fatty solids 9·12 per cent., fat 3·25 per cent.
Bottom tap: ,, ,, 9·03 per cent., ,, 3·25 per cent.
Initial temperature of milk .. .. 86° F.
Final temperature of milk .. .. 75·2° F.
Dairy temperature at 5.5 a.m. .. .. .. 41·9° F.
Dairy temperature at 7.30 a.m. .. .. .. 44·6° F.

TABLE IX.

Date:—26th November, 1929.

Time. a.m.	"Top milk." Non-fatty solids. Per Cent.	Fat. Per Cent.	"Top tap." Non-fatty solids. Per Cent.	Fat. Per Cent.	"Middle tap." Non-fatty solids. Per Cent.	Fat. Per Cent.	"Bottom tap." Non-fatty solids. Per Cent.	Fat. Per Cent.
5.15	9·08	3·32	9·16	3·22	9·08	3·25	8·90	3·32
5.35	8·96	3·42	9·00	3·25	9·07	3·25	9·00	3·20
5.55	8·97	3·67	9·10	3·32	8·99	3·20	9·08	3·07
6.15	8·92	4·65	9·00	3·45	8·99	3·20	9·05	3·11
6.35	8·87	5·85	9·02	3·30	8·97	3·22	8·93	2·97
6.55	8·70	7·57	8·97	3·20	9·01	3·05	9·04	2·72
7.15	8·85	8·55	9·03	3·05	8·93	2·82	9·06	2·55
7.35	8·70	10·05	9·05	2·95	8·96	2·70	9·05	2·40
7.55	8·55	10·95	9·08	2·90	9·11	2·57	9·05	2·30

8.00—Last samples of series taken from top of churn by dipping and from bottom tap after plunging contents of churn:

Top milk: Non-fatty solids 9·00 per cent., fat 3·05 per cent.
Bottom tap: ,, ,, 9·03 per cent., ,, 3·00 per cent.
Initial temperature of milk .. .. 86° F.
Final temperature of milk .. .. 77° F.
Dairy temperature at 5.15 a.m. .. .. .. 50·9° F.
Dairy temperature at 7.55 a.m. .. .. .. 49·1° F.

The main fact which emerges from these figures is that on standing undisturbed for a period of twenty minutes the fat in "fresh" milk unquestionably begins to rise to the surface, the rate being much more marked with cooled than with uncooled milk, while in an hour and twenty minutes the rise is much more definite; proof of this statement is found in the composition of the samples taken from the churn by "dipping" and by drawing from the taps, but the presence of a cream layer can also be detected by inspection of the contents of a churn.

It is true that when portions of uncooled milk are poured from a churn at intervals of twenty minutes there is, apparently, no very marked rise of fat to the surface when the fat-content of the several portions is considered, but this is due to phenomena in which temperature, surface tension and what, for want of a better expression, may be termed the "fluidity" of the aggregate of fat globules which go to form the layer known as cream, all play a part.

When uncooled milk is poured from a churn under the conditions which have been observed throughout this investigation, the "spread" of the cream layer over the whole superficial area of liquid contained in the churn bears some fairly constant relation to the bulk of the milk, and is more or less proportional to the fat-content of that milk.

The rise of fat is gradual, to judge from the results I give, but from the very nature of the fat globules, as compared with the serum in which they are diffused, it must begin the moment movement of milk ceases; when the first portions are poured from a considerable volume of milk in a churn which has been just filled, there is a lower concentration of fat in the upper layers than at subsequent periods,

but as the concentration of fat increases in the upper layers, the tilt of the churn when pouring off consecutive portions becomes greater as the volume of milk in the churn decreases; consequently there is exposed a greater superficial area of liquid and a greater dispersion of the individual particles of fat floating on the surface, with the result that there is no very appreciable alteration of the fat-content of the various portions or in the residue finally remaining in the churn, a result which might be anticipated if the above explanation is correct.

There is no evidence of acceleration in any marked degree in the rise of fat globules, but there is ample proof that the effect of the rise in the upper layers is an accumulation of fat, while in the other regions of the churn there is a replacement of the globules, at a fairly uniform rate, from the bottom upwards.

EFFECT OF ALTERATION OF MILKING HOURS ON FAT OF MILK.—Another matter of interest which has received attention and which bears on the variation of the fat-content of milk is the effect produced by the alteration of milking hours. It is well known that when cows are holding their milk there is a tendency for the fat to become re-absorbed, but it has been claimed that the phenomenon results in the increase or decrease of the fat-content according to the lengthening or shortening of the time interval between an evening and a succeeding morning milking, such being its operation that it is capable of being reduced to an exact mathematical expression from which a constant, represented by a diminution of 0·24 per cent. of fat for every hour by which the interval between milkings is lengthened has been evolved; the converse is also said to operate.

Two series of tests were made, in the first of which the intervals between evening and succeeding morning milkings were extended by one hour and forty-eight minutes, and one hour and fifty minutes, with a four-day interval after the alteration of the milking period; the result was that there was a decrease of 0·26 per cent. and 0·28 per cent. of fat in the respective samples (Table X), but according to "Collin's Rule," as it is called, there should have been decreases of 0·42 per cent. and 0·44 per cent.

TABLE X.

Sample No.	Date.	Time.	Non-fatty solids. Per Cent.	Fat. Per Cent.	Interval between milking in hours and minutes.
374	27/3/29	3 p.m.	8·83	3·87	Start.
374A	28/3/29	5.36 a.m.	8·94	3·48	14 hrs. 36 mins.
375	28/3/29	2.45 p.m.	8·64	4·55	9 ,, 9 ,,
376	29/3/29	5.37 a.m.	8·88	3·46	14 ,, 52 ,,
377	29/3/29	2.40 p.m.	8·80	4·27	9 ,, 3 ,,
385	30/3/29	7.20 a.m.	8·90	3·20	16 ,, 40 ,,
386	30/3/29	2.40 p.m.	8·81	5·07	7 ,, 20 ,,
387	31/3/29	5.40 a.m.	8·95	3·65	15 ,, 0 ,,
388	31/3/29	2.45 p.m.	8·88	4·12	9 ,, 5 ,,
389	1/4/29	5.45 a.m.	8·78	3·65	15 ,, 0 ,,
390	1/4/29	2.40 p.m.	8·76	4·20	8 ,, 55 ,,
391	2/4/29	5.50 a.m.	8·56	3·70	15 ,, 10 ,,
392	2/4/29	2.30 p.m.	9·37	4·65	8 ,, 40 ,,
393	3/4/29	7.30 a.m.	8·90	3·42	17 ,, 0 ,,

In the second series (Table XI) it was arranged to extend the intervals between evening and succeeding morning milkings, and also between morning and succeeding evening milkings; the first extension of half an hour apparently resulted in a drop of 0·58 per cent. of fat in an evening milk, while a further lengthening of an hour and twenty-five minutes brought about no change, but the succeeding morning milk showed a drop of 0·28 per cent. of fat, although the interval between it and the previous evening milking was less by an hour and three-quarters than the normal interval; the next morning milk showed an increase of 0·18 per cent. of fat, although the interval was here prolonged by three hours and a half; while on the morning following the fat was 0·03 per cent. lower—for all practical purposes the same—in spite of the interval having been restored to the normal within all but a few minutes.

TABLE XI.

Date.	Time.	Non-fatty solids. Per Cent.	Fat. Per Cent.	Intervals between milking in hours and minutes.
22/7/29	6.00 a.m.	8·83	3·10	Start
22/7/29	2.45 p.m.	8·65	4·40	8—45
23/7/29	6.05 a.m.	8·78	3·17	15—20
23/7/29	2.40 p.m.	8·94	4·50	8—35
24/7/29	5.40 a.m.	8·97	3·20	15—00
24/7/29	2.45 p.m.	8·74	3·92	9—05
25/7/29	5.55 a.m.	9·06	3·05	15—10
25/7/29	4.25 p.m.	8·95	3·92	10—30
26/7/29	5.50 a.m.	9·18	2·77	13—25
26/7/29	2.45 p.m.	8·79	4·17	8—55
27/7/29	7.40 a.m.	9·02	2·95	16—55
27/7/29	2.35 p.m.	8·70	5·52	6—55
28/7/29	6.10 a.m.	9·01	2·92	15—35
28/7/29	3.00 p.m.	8·88	3·90	8—50

In the light of the above facts is it a reasonable thing to say that this rule embodies a fundamental principle? Is not, indeed, the whole process of the production of milk by mammals a physiological function which is not, and cannot be, subject to mathematical control; there can be no doubt that if the balance which is automatically maintained is upset, whatever the cause, then variations do occur in the fat-content, but it does not by any means appear to be established that a return to normal conditions means a return to normal quality, and by normal quality I mean the quality of milk which is produced from day to day by the particular collection of cows which has been subjected to alteration of the conditions to which it is accustomed.

Furthermore, the champions of this principle appear to ignore the effect of a factor upon which so much stress is laid on other occasions, and that is the day-to-day variations in fat-content; how is this influence eliminated when the attempt is made to equate the alteration in milking hours and the increase or decrease of fat which results therefrom?

EFFECT OF STANDING ON SOLIDS-NOT-FAT.—So far I have confined my attention to the question of fat, but there is a point connected with solids-not-fat to which I should like to call attention.

It has been said that if a quantity of milk were allowed to stand at rest for some time in a vessel such as the tapped churn which I used in some of my investigations, there would be not only a rise of fat to the surface of the milk, but there would be also a settlement of solids-not-fat, so that if a sample were drawn from the central region of the vessel, the deficiency would be sufficiently great to convey the impression that there had been an addition of water; I, myself, heard this suggestion made in an attempt to explain the low figure for solids-not-fat in a certain sample of milk, although, I am glad to say, it was a lawyer, and not a chemist, who made it.

In order to examine the possibility of any marked separation of solids-not-fat taking place, the figures obtained in the tapped churn experiments have been calculated to the basis of the fat-free milk, and the solids-not-fat so found have been plotted against the time intervals at which samples were drawn.

The resulting curves are remarkable and certainly do not indicate that the highest proportion of solids-not-fat is contained in the bottom part of the milk, although in the first test this does appear to be so, but in that case it will be remembered that glass tubes were used inside the churn, whilst in the other three the solids-not-fat from the bottom-tap samples all occupy more or less of an intermediate position, and do not show a very marked rise at the end of the experiment; and certainly in no case is the increase one which would suggest that there had been an addition of water, while the samples drawn from the middle tap, in which a marked deficiency of solids-not-fat would be expected, fail to bear this out.

A noticeable feature is the marked increase of solids-not-fat which appears to accompany the accumulation of fat in the upper layers, and this is most noticeable in the Table IX when the figures are plotted after correcting for fat; it occurred to me that the difficulty of drying milk solids containing a rather high fat might account for this, and I therefore repeated the determination, using a smaller quantity of milk and diluting with water before evaporation, in order to obtain a thinner film, in the samples of "top milk" taken at 7.15, 7.35 and 7.55 a.m., with the result that slightly lower figures were found, but not so much lower as to affect the direction of the curve.

At the moment it has not been possible to make an attempt to arrive at the true explanation of this, and therefore I do not intend to enter into speculation as to the cause, but I felt compelled to draw attention to the fact.

The Examination of Milk for Tubercle Bacilli.

A Survey of Experience and Results.

By D. R. WOOD, F.I.C.

(*Read at the Meeting, May* 7, 1930.)

HAVING examined some fifteen hundred samples of milk for tubercle bacilli, I thought that some account of the results obtained and of lessons learnt might not be without interest to others.

The large majority of the samples were from the milk of herds of cows in this county; a systematic examination of these herds has been undertaken with the object of finding and eliminating cows giving tuberculous milk. Some of the samples were examined under the provisions of the Tuberculosis Order and some for private individuals, but these will not concern us much in this paper.

The following table (Table I) shows the number of herds whose milk was tested by me and the number in which tubercle bacilli were found in the four years 1926 to 1929; for comparison, results obtained with mixed milk samples taken from churns in other localities are given in Table II.*

TABLE I.

TUBERCLE BACILLI IN MILK OF SOMERSET HERDS.

Year.	Number of herds tested.	Number of herds giving tuberculous milk.	Percentage of herds giving tuberculous milk.
1926	180	4	2·2
1927	230	5	2·2
1928	273	6	2·2
1929	317	8	2·5

* The annual percentages of herds in this county giving tuberculous milk have admittedly been calculated from a rather small number of samples. In the first year little confidence was placed in the figure obtained, but when an almost identical figure was obtained for each of the three following years and the total number of samples for the four years amounted to 1,000, there was every indication that a figure between 2 and 3 per cent. for the tuberculous herds was reliable. Since this paper was written information has come to hand of the results of the London County Council for 1928 and 1929 for samples of milk coming from Somerset, showing 3·13 per cent. positive, the number of samples examined being 256. This is in very fair agreement with my results. The percentage containing tubercle bacilli from another county was 9·25.

TABLE II.

TUBERCLE BACILLI IN MIXED MILK SAMPLES FROM VARIOUS LOCALITIES.*

Locality.	Period.	Mean percentage containing tubercle bacilli.	Maximum and minimum annual percentages.
Liverpool (County samples) ..	1919–1927	7·4	3·1–10·45
,, (Town samples) ..	do.	6·4	2·5–15·2
Aberdeen	1922–1927	5·5	3·0– 8·3
Salford (Cheshire milk) ..	1920–1927	11·5	8·9–14·2
,, (Lancashire milk) ..	do.	3·35	1·3– 8·0
Birmingham	1918–1927	7·5	3·5– 9·6
Newcastle-upon-Tyne ..	do.	4·95	2·9– 8·0
London County Council ..	do.	5·3	3·1– 7·8
Manchester	do.	10·6	5·3–14·9
Monmouthshire	1927	1·4	—

The percentage of Somerset herds giving tuberculous milk is remarkably constant, and is decidedly lower than that of other localities for mixed milk samples. The two classes of samples, however, are not really comparable, for with mixed milk samples, the milk is frequently mixed in bulk in large tanks and many churnfuls may be contaminated by the milk of a single cow.

Table III gives a summary of the results of investigation of 30 Somerset herds whose milk was found to contain tubercle bacilli.

RELATIVE VALUE OF MICROSCOPICAL AND BIOLOGICAL TESTS.—It was realised from the outset that it was perfectly useless to attempt to find tubercle bacilli in the milk of herds by microscopical examination (the milk of every herd was centrifuged for one hour at a speed of 3,500 R.P.M., the deposit from 100 c.c. suspended in 1 c.c. of sterile water and inoculated into a guinea pig)—but it was not quite realised how little one can rely upon finding tubercle bacilli microscopically in the milk of single cows. In the investigation of the first herd (No. 1) the milk of each cow was examined microscopically only, and no tubercle bacilli were found. With No. 3 tubercle bacilli were found microscopically after using a concentration method. With No. 4 tubercle bacilli were not found microscopically in the first examination of the milk of two cows containing these bacilli, but only upon subsequent examination of other samples, and then only by the use of a concentration method; while of the remaining 17 samples from single cows, undoubtedly shedding tubercle bacilli in their milk, only 4 showed these bacilli microscopically; or, counting the 3 additional samples mentioned in Table III in which tubercle bacilli were found later microscopically, the total number becomes 23, and the number showing tubercle bacilli microscopically 8, or 37·5 per cent. Savage (*loc. cit.*, p. 121) gives a tabular summary of the relative frequency with which tubercle bacilli have been found microscopically (1) by me ("Somerset"), (2) in the county of Wiltshire, and (3) by Mr. A. T. R. Mattick of the Reading Laboratory. As he indicates, however, the higher proportion which I found positive microscopically was due to the inclusion of special samples from very advanced cases. Table IV

* Taken from *The Prevention of Human Tuberculosis of Bovine Origin*, by W. G. Savage.

TABLE III.

INVESTIGATIONS OF SOMERSET HERDS GIVING TUBERCULOUS MILK.

Serial Number of Herd.	*Date of examination.*	*No. of cows in Herd.*	*No. of cows whose milk was separately tested.*	*No. of cows showing T.B. in milk.* Micro-scopically.	By inoculation.	*Outcome of investigation.*	*Post-mortem findings by Veterinary Surgeon.*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	Mar. 1926	25	8	0	0		
			17	0	0		
2	May ,,	22	22	0	0	F	
3	Sept. ,,	?	1	1	not inoculated	S	General t. and udder t.
4	,, ,,	?	2	0	2	S	(1) do. do. (2) do. and supra-mammary t.
5	Jan. 1927	20	5	0	0	F	
6	Feb. ,,	32	4	0	0	F	
7	July ,,	65	6	0	1	S	General t. and udder t.
8	Aug. ,,	27	3	0	0	S_{cl}	Cow (dry) found with general t. and thickened quarter.
9	,, ,,	46	3	0	1	S	Advanced t. and udder t.
10	,, ,,	15	2	1	not tested	S	do. do.
11	July ,,	46	3	0	0	S_{cl}	Cow killed under T.O.
12	Nov. ,,	16	4	0	0	S_{cl}	,, ,, ,, ,,
13	June ,,	63	5	0	1	S	Advanced t. and udder t.
14	Nov. ,,	20	2	0	0	S	
	Mar. 1928		3	0	0		
	Aug. ,,		19	0	1		(1) Udder tuberculosis.
				0	1		(2) Tuberculosis lesions but none in udder.
15	May 1928	15	3	0	0	F	
16	,, ,,	20	2	0	0	S_{cl}	Dry cow killed under T.O. and t. udder found.
17	June ,,	27	3	0	1	S	Advanced t. but no evident udder t.
18	July ,,	36	6	0	0	S	
	Feb. 1929		*8	0	1		Advanced t. and udder t.
				0	1		do. and slight udder t.
19	Sept. 1928	21	2	0	1	S	Advanced t. and udder t.
20	Sept. ,,	47	6	0	0	S	Udder t. and other lesions.
			*8	1	1		
			*4	0	0		
21	Feb. 1929	?	2	1	1	S	Advanced t. and udder t.
22	May ,,	?	4	0	0	F	
23	Aug. ,,	?	3	0	2	S	(1) Advanced t. no udder t. evident (2) ,, udder very healthy.
24	,, ,,	?	1	0	1	S	General t. and udder t.
25	,, ,,	32	2	0	1	S	No P.M.
26	,, ,,	35	3	1	1	S	t. of udder and other lesions.
27	,, ,,	29	3	0	0	S_{cl}	Cow sold and killed and found to have advanced t.
28	Oct. ,,	26	2	0	0		Further investigation in hand.
29	Nov. ,,	30	1	0	0		
30	Dec. ,,	32 tested in sections					

S indicates success in detecting at least one cow yielding tuberculous milk; S_{cl} indicates that this was accomplished by clinical examination; and F indicates failure in detecting any such animal.

t. stands for abbreviation for tuberculosis, and T.O. for Tuberculosis Order.

* These cows were selected from the herd by the examination of the milk of the herd in sections, the milk of each cow of the sections showing tubercle bacilli was then tested separately.

NOTES.—Herd No. 1. These were microscopical examinations only.
No. 2. Milk of herd re-examined later and found negative.
Nos. 4 and 14 (1). Tubercle bacilli found microscopically in later samples from the three cows.
No. 11. Udder macroscopically not tubercular.
No. 12. do. do. do.
No. 15. Milk of herd re-examined and found negative.
No. 22. Milk of herd examined in sections and all found negative.

reproduces Savage's table, excluding these very advanced cases (many of which did not require bacteriological diagnosis) and bringing results up to date. It will be noted that the results are in very good agreement, and one can reasonably assume that in only one case in three at the best evidence will be obtained microscopically of the presence of tubercle bacilli.

TABLE IV.

Area.	Positive by inoculation.			Negative by inoculation.		Percentage + by inoculation and — microscopically.
	+ microscopically.	— microscopically.	doubtful.	+ microscopically.	doubtful microscopically.	
Somerset	8	15	0	0	5	62·5
Wilts.	19	22	0	2	0	53·6
Reading Lab.	24	50	0	—	—	67·6
Total	51	87			Average	63·0

THE CYTOLOGY OF TUBERCULOUS MILK.—The great advantage of finding tubercle bacilli microscopically, when possible, is the saving of time it effects. When it is discovered that a herd is giving tuberculous milk it is impossible to stop the sale of milk pending the investigation of the milk of the individual cows; this takes four to five weeks by guinea pig inoculation. But, as microscopical examination cannot be relied upon, attention was giveu to the cytology of tuberculous milk. One expects to find an increase of cells in tuberculous milk from single cows; this increase, however, may be very slight and more than compensated by the variation in cellular contents under normal conditions of the cow. On one occasion samples of milk were collected separately from the four quarters of a cow whose milk was positive by inoculation, and the following results were obtained:

Quarter:	Right fore.	Left fore.	Right hind.	Left hind.
Cells per c.c.	50,000	5,000	400	20,000
Tubercle bacilli microscopically	absent	absent	present	absent

On another occasion the milk of twelve cows of a herd giving tuberculous milk was examined separately, special attention being given to the increase of cells, and the following results were obtained:—

Cow No.	Cells.	Result of inoculation tubercle bacilli.
1	Normal in number	absent
2	Increased in number	do.
3	,, ,, ,, ...	do.
4	Slightly increased in number	do.
5	Normal in number	present
6	,, ,, ,, ...	absent
7	,, ,, ,, ...	do.
8	Increased in number	present
9	Normal ,, ,, ...	absent
10	,, ,, ,, ...	do.
11	Increased ,, ,, ...	do.
12	,, ,, ,, ...	do.

Attention was also given to the predominant type of cell present in tuberculous milk. Generally speaking, the predominant cells in acute infections are the polymorphonuclear leucocytes, and in the chronic infections such as tuberculosis the lymphocytes. One would expect therefore a predominance of mono-nuclear cells in tuberculous milk, but this is far from being the case. Buchanan describes a large mono-nuclear cell as characteristic of tuberculous udders, and uses Papenheim's stain, but my experience with this stain and Jenner's stain was very disappointing.

THE LIMITATIONS OF VETERINARY INSPECTION*.—When a herd giving tuberculous milk had been discovered, it was considered reasonably certain at the outset that by careful veterinary inspection of every cow in the herd those with tuberculous udders would be detected, or at least suspected; in fact, that such pleasing success in spotting the right cows as we had in cases three and four would be common experience. Comparison of the figures in the 4th and 6th vertical columns of Table III—cows suspected and cows with tuberculous udder—shows that this was far from being the case. The cow's udder is a bulky organ, and the lesions may be very slight; in fact, pin-points, as was shown in case 24, where the opened udder appeared quite normal without a hand lens. Three cases were particularly intractable, Nos. 14, 18 and 20. With No. 14 a herd of 20 cows was three times reported from Bristol to contain tubercle bacilli, and each time every cow was inspected by a veterinary surgeon. Two, three and four cows on the respective occasions were suspected and their milk submitted for examination, but with negative results both microscopically and by inoculation. Finally the milk of every cow was tested and tubercle bacilli were found in the milk of two. Even then the veterinary surgeon was so convinced that one of these cows was healthy that the examination was repeated, and tubercle bacilli again found before the cow was destroyed.

The tuberculin test merely indicates whether or not a tubercular lesion is present. About 40 per cent. of cows give a positive reaction, and the test is of very little use by itself in deciding whether a cow has a tuberculous udder or even comes under the Tuberculosis Order.

THE EXAMINATION OF THE MILK OF HERDS IN SECTIONS.—With No. 18, a herd of 36 cows, at the first inspection six cows were considered possibly responsible and their milk was examined, but with negative results. The herd was then divided into 5 sections and the milk of each section tested. Tubercle bacilli were found in the milk of one section, representing 8 cows. The milk of these 8 cows was then tested separately, and tubercle bacilli were found in the milk of two.

The method of examining the milk of a herd in sections was adopted again in case No. 24, and tubercle bacilli were found in two sections. Every cow's

* It is only fair to say that such was my experience up to the time of writing, when only part-time veterinary surgeons were employed. The county has since then appointed a whole-time officer specialised in the work, and it appears probable that my estimate of the value of inspection will, in future, be distinctly modified.

milk of these two sections was subsequently tested, the cow responsible for the presence of T.B. in the milk of one of these sections only was discovered, the four cows of the second section all giving negative results by inoculation.

One is driven to the conclusion from these three instances that the only logical and safe procedure is either to test the milk of every cow of the herd separately or in groups of about six, and then every cow of the positive group or groups, for, if the herd is merely inspected, no cow giving tuberculous milk may be found amongst the animals suspected by the veterinary surgeon, and even if such a cow is found there is no guarantee that another cow may be not shedding tubercle bacilli as well.

REVIEW OF THE SUCCESS OR FAILURE OF INVESTIGATION OF HERDS GIVING TUBERCULOUS MILK.—Column 7 of Table III shows the outcome of each investigation at a glance. The letter S indicates success in detecting at least one cow yielding tuberculous milk, S_{cl} indicates that this was accomplished by clinical examination, and F indicates failure in detecting any such animal. It will be noted that there were twenty-one successful investigations and five failures, excluding the first case when my technique was in a somewhat experimental stage and microscopical examination only was made of the majority of samples tested. Of the five failures, numbers 5 and 6 were quite probably due to some of the inoculated guinea pigs dying prematurely. In numbers 2, 15 and 22 a possible explanation is that the milk of the original sample was infected from the excreta, for the milk of every cow was tested either individually or in groups and the full period of incubation of inoculated guinea pigs was given. But there are two other possibilities: the possibility of failing to get tubercle bacilli from the udder of a cow in the early stages of infection, through insufficient massage in milking, and failure to keep track of all the cows contributing to the original sample. In compliance with the Milk and Dairies Order, 1925, 24 hours' notice must be given to the farmer of the intention to inspect his herd; this gives a dishonest farmer the opportunity of disposing of a cow with a suspicious udder, which is very undesirable; no such opportunity is given in Scotland. It will be noted that a later examination of the milk of two of these five herds gave negative results.

Such, then, are the results obtained by the examination of the milk of 1000 herds. There are some 7000 herds in this county; at the present rate therefore 28 years will be required to examine the milk of every herd, by which time of course every cow will be replaced. This cannot be regarded as an adequate measure for the elimination of tuberculous milk; if this is to be achieved something like seven times as many examinations should be made.

SOMERSET COUNTY LABORATORY.

The Determination of Platinum in Platinum Ore.

By W. R. SCHOELLER, Ph.D.

In a recent review (Analyst, 1930, 354), I had occasion to oppose the view that the use of ammonium chloride as a reagent in the analysis of platiniferous materials is only "justified by the demands of commercial work for a rapid method of approximate accuracy," yielding serviceable results "only by accidental compensation of large errors." While familiar with the disadvantages of ammonium chloride (which I discussed in the Analyst, 1926, **51**, 392), I expressed the opinion that the salt, in practised hands, is a most serviceable and, possibly, indispensable reagent in precious metal work.

The present paper is intended to substantiate this view by briefly explaining the modified procedure which I have found to give reliable results in the analysis of native platinum and high-grade platinum concentrates. Low-grade ores, the assay of which involves fusions with litharge and fluxes, are not considered in this paper. I believe that the "chlorine process," as it may be termed for conciseness, removes the most serious objection raised against the use of ammonium chloride, and simplifies a difficult problem of mineral analysis.

Principle of Chlorine Process.—The usual procedure for the analysis of platinum minerals is still more or less modelled on that of Deville and Debray (1859). It aims at the quantitative precipitation of the platinum as ammonium chloroplatinate by working in a solution saturated with ammonium chloride. Under those conditions the recovery is practically complete, but the precipitate is impure, as it adsorbs rhodium, palladium, and gold if present. Adsorption of the common metals (chiefly iron) is less pronounced. If the precipitate, after ignition and reduction, is weighed as (Pt+Ir), a positive error is obtained, the magnitude of which is ordinarily one-quarter to one-half per cent. Hardly any reference has been made in the published methods to this error, still less to the means for obviating it.

The principle underlying the chlorine process is simple, but of practical importance. If a substance cannot be precipitated quantitatively in one operation without undue contamination, it may be feasible to obtain it in two fractions. The first or major fraction should be pure. The minor fraction completes the precipitation; it is not pure, but, if sufficiently small, renders the error negligible for practical purposes.

In the case under discussion, the simplest way to apply the above principle appears to be by re-treatment of the ammonium chloroplatinate precipitate, the removal of the greater part of the minor constituents and of the large amount of iron present having been achieved by the primary precipitation, filtration, and

washing. Ignition of the precipitate, solution of the sponge in *aqua regia*, evaporation with hydrochloric acid to expel nitric acid, and re-precipitation with excess of ammonium chloride as before is not only tedious but ineffective, as the second precipitate may not be quite pure, and part of the adsorbed rhodium becomes insoluble, together with most of the iridium. Simple re-crystallisation from hot water answers quite well for small amounts of platinum, but in the case of rich ores the sparing solubility of the precipitate renders this mode of working impracticable on account of the large volume of water required. In that case the ammonium chloroplatinate may be destroyed by digestion with hot *aqua regia*, but this again is a protracted operation, the crystalline precipitate exposing but a small surface to the action of the acid. Now the modification I have introduced in platinum analysis consists in dissolving large quantities of ammonium chloroplatinate—or rather, re-converting it into chloroplatinic acid—by the action of a current of chlorine upon the suspension of the precipitate in boiling water. This simple and rapid process will now be described.

THE PROCESS.—The ammonium chloroplatinate precipitate obtained in the usual manner is collected, thoroughly washed with half-saturated ammonium chloride solution, then twice with cold water, which frees it from most of the ammonium chloride. It is then rinsed back into the precipitation vessel (a 400 c.c. squat beaker), and the filter completely cleaned, with boiling water.

Major Platinum Fraction.—The liquid (about 150 c.c. for 1 grm. of Pt.), slightly acidified with hydrochloric acid, is now vigorously boiled and kept stirred by means of a glass tube through which a brisk current of chlorine is passing. The precipitate dissolves readily, usually in a few minutes. When solution has taken place the chlorine current is interrupted, the tube washed down with hot water, and the solution placed on a hot plate for evaporation after addition of just enough ammonium chloride to combine with the platinum (6 c.c. of 10 per cent. solution for 1 grm. of Pt.). When the bulk has been reduced to about 20 c.c., the solution is left to cool and diluted with an equal bulk of half-saturated ammonium chloride solution. After standing for a few hours the coarsely-crystalline precipitate, P^1, is collected, washed with the same ammonium chloride solution, and transferred to a tared porcelain crucible; it is ignited with the usual precautions, and weighed. It contains the great bulk of the platinum and iridium free from other metals.

The filtrate and washings from P^1 are added to those from the primary ammonium chloride precipitate, and evaporated with a crystal of sodium chlorate until a crust of ammonium chloride is formed. The small, dark precipitate, P^2, which contains the balance of the platinum and iridium and, generally, a little palladium, is collected, washed with ammonium chloride solution, strongly ignited, reduced, and extracted with dilute *aqua regia* (1: 3 water). The insoluble residue (a little iridium) is collected, washed, ignited, weighed, and added to P^1, which is then wrapped in lead foil and heated in a carbon crucible for an hour at 1000° C. This operation, followed by extraction with acids (*vide infra, Iridium Determination*) gives the weight of the iridium, and platinum in P^1 by difference.

Minor Platinum Fraction. The filtrate resulting from the *aqua regia* extraction of the ignited precipitate P^2 normally contains the balance of the platinum. It is evaporated to dryness, then twice with hydrochloric acid, the residue taken up with a little water acidulated with hydrochloric acid, and the solution precipitated with ammonium chloride. The resulting precipitate, P^3, is converted into platinum as usual; it is not so pure as P^1, but, being small, its weight after ignition can be added to that of the major fraction without causing an appreciable error.

Testing for Complete Precipitation.—It remains to ascertain the completeness of the platinum precipitation. To this end the combined filtrates from P^2 and P^3 are exhaustively treated with pure granulated zinc. The precipitate is collected, washed with acidulated water, and returned to the beaker; it is then digested with hot dilute *aqua regia* (1:7 water). The insoluble portion of the rhodium, if any, is filtered off and the filtrate treated exactly as the one from which the minor fraction was obtained, *i.e.* double evaporation with hydrochloric acid and precipitation with ammonium chloride. If a precipitate is obtained, it is ignited and weighed. It should be so small that no appreciable error is incurred by addition of its weight, even if only 90 per cent. pure, to the platinum result.

NOTES ON THE PROCESS.—The chlorine process has been used for a large number of analyses of platinum minerals with such satisfactory results that I have now adopted it as the regular method of analysis. Three or more determinations are made on 25 c.c.-portions of solution obtained by dissolving 30 to 35 grms. of ore in *aqua regia*, filtering, and diluting to 500 c.c.; nitric acid is expelled by two evaporations with hydrochloric acid. Gold, if present, is next removed by cautious addition of sulphurous acid to the hot, acid solution; after ignition it should be yellow, not discoloured, otherwise it is dissolved and re-precipitated. The combined filtrates are evaporated, oxidised with a little nitric acid, and again evaporated with hydrochloric acid. The residue is dissolved in 5 c.c. of the same acid and 20 of hot water, and precipitated by the gradual addition of an equal bulk of half-saturated ammonium chloride solution. After standing for some hours or overnight the precipitate is collected, washed with the ammonium chloride solution, then twice with cold water, after which it is ready for the chlorine process. The final platinum results should, and generally do, agree within 0·001 grm.

Palladium Test.—One of the weighed platinum-iridium sponges may be tested for palladium by solution in *aqua regia*, evaporation, dilution, filtration, and addition of dimethylglyoxime. I may add that this test has invariably given negative results in spite of its delicacy, and although quantities of palladium as high as 0·08 grm. have been originally present.

Iridium Determination.—Duplicate determinations of iridium by the lead fusion method are made on the other two sponges. The lead button is boiled with 15 per cent. nitric acid, the filtered extract precipitated with sulphuric acid, and the lead sulphate filtered off by suction and discarded; the filtrate is boiled down till the nitric acid is expelled. The residue from the nitric acid extraction is

treated with 15 to 20 c.c. of strong sulphuric acid (including the acid left after evaporation of the nitric acid extract) and 5 grms. of ammonium sulphate; after expulsion of the water, the temperature is raised so that the acid volatilises freely. After cooling and dilution, the solution is filtered; lead sulphate in the residual platinum is extracted with ammonium acetate solution, and the platinum dissolved in dilute *aqua regia* for the determination of iridium by known methods.

Rhodium Test.—The filtered sulphuric-acid extract may be tested for rhodium. It is precipitated exhaustively with pure zinc, the precipitate (if any) collected, ignited, reduced, mixed with sodium chloride, and heated in a Rose crucible in a current of chlorine. The fused mass, dissolved in a few drops of water, yields a rose-red solution if rhodium is present. An orange tint may be due to minute amounts of platinum (palladium); in such a case, addition of a little ammonium chloride (dimethylglyoxime) and filtration will bring out the characteristic colour of any chlororhodite present. Whilst not advancing the chlorine process as a quantitative separation of platinum from rhodium in any proportions, I have found that its application in platinum ore analysis (maximum amount of rhodium observed, 0·015 grm. to 1·06 grm. of Pt) gave a final platinum precipitate in which no rhodium could be detected by the above test.

Coarsely-crystalline ammonium chloroplatinate, slowly deposited from solutions containing a little rhodium and a certain excess of ammonium chloride (a condition not obtaining in the chlorine process) is known frequently to assume a more or less pronounced green colour (Deville and Stas's *vert de platine*); it may be as deep as emerald green, as I have repeatedly observed. Wunder and Thüringer's belief that the green colour may be due to the presence of iridous chloride (*Z. anal. Chem.*, 1913, **52**, 744) is erroneous, for the solutions from which I obtained the green crystals were always rhodiferous but iridium-free. I am satisfied that the green colour is due to rhodium, in quantity well below 2 per cent. of the precipitated platinum. When, on the other hand, a platinum solution containing little rhodium is precipitated rapidly with ammonium chloride, the resulting precipitate is always yellow, yet never rhodium-free. Until proof of the contrary, it may be assumed that the yellow precipitate contains adsorbed rhodium, whereas the green crystalline material consists of mixed crystals or a solid solution.

Incidentally, I may mention the existence of what may be regarded as a solid solution of rhodium chloride in lead chloride. When a chloride solution containing a very small amount of rhodium and a little lead is evaporated to crystallisation of lead chloride, the resulting crystals will be of a fine, delicate pink or rose-red colour. The pink crystals have been observed to deposit from solutions containing a large excess both of gold and of platinum, their formation proving the presence of less than 0·1 per cent. of rhodium.

The Colorimetric Determination of Manganese in Biological Material.

By MARION B. RICHARDS, D.Sc.

THE possible importance of manganese as a factor in animal metabolism has been indicated by various authors.[1] With a view to studying more closely the function of this element in nutrition, it has been necessary to work out a technique for the accurate and rapid determination of small amounts of manganese in biological material, particularly in that of animal origin.

While various methods of determining manganese, as described in the literature, are fairly satisfactory under the conditions given, investigation has shown that in dealing with such minute amounts as are liable to occur in animal tissues, certain precautions are necessary which seem to have been overlooked by most workers.

The persulphate method of oxidation of the manganese to permanganate, in readiness for the colorimetric comparison, has been widely used since its introduction by Marshall[2] in 1901, and Wester[3] has worked out very carefully the conditions under which it can be used for the determination of very small amounts of manganese in such materials as the ash of plants. The periodate method of Willard and Greathouse[4] seems preferable to that of Marshall in that it requires no catalyst, and is claimed by its authors to be free from various faults of previous methods. It was originally applied to the determination of manganese in steels and iron ores, and the present paper seeks to show, as Wester has done for the persulphate method, the conditions under which it may be used for the determination of minute amounts of manganese in organic material. It will be seen that, due regard being paid to one or two details in the procedure, the periodate method is preferable to the persulphate method in determining small amounts of manganese, just as it is preferable in the estimation of larger amounts.

PROPOSED MODIFICATIONS.—It has been found that in the application of the periodate method to the determination of traces of manganese, two points in particular require attention:—(1) The acidity of the solution to be oxidised, and (2) the removal of chlorides.

I. ACIDITY OF THE SOLUTION DURING OXIDATION.—(*a*) *Danger of Excess Acidity.*—Willard and Greathouse, in describing their method, emphasise the danger of having insufficient acid present, and state that a very large concentration of acid does no harm. This is no doubt true for the amounts of manganese dealt with in their tests, but the lowest amount they used was 2·5 mgrm. of manganese, a quantity much beyond the amount present in a workable sample of most organic materials.

If the amount of manganese present is very small, *e.g.* 0·005 mgrm. or less, as quite frequently occurs in the analysis of animal organs, the danger lies in excess of acidity, rather than in its insufficiency. In such cases an acidity of 15 per cent. sulphuric acid (*i.e.* 15 c.c. of concentrated acid per 100 c.c. of solution) is the maximum permissible. An acidity of 20 per cent. is sufficient to prevent the full colour from developing, or to cause it to fade rather rapidly, the solution generally assuming a yellow tint. Moreover, it has been found that if the colour has been destroyed by excess of acidity, it cannot be fully restored by subsequent dilution of the solution.

In a recently published paper Skinner and Peterson[1] report that in a few instances during the oxidation with periodate, the permanganate colour faded as the volume was reduced—a difficulty which they overcame by the addition of a few drops of syrupy phosphoric acid to the boiling solution. As their paper was published after the present tests of the method were satisfactorily concluded, I have not tested whether a coloration that has faded from excess of acidity can be restored by means of phosphoric acid, but it may be stated that with the acidity carefully restricted, no such fading has been experienced.

(*b*) *Rate of Colour Development at Different Acidities.*—Willard and Greathouse state that increase of acidity increases the rate of colour development, and while this is true for the concentrations of 15 per cent. sulphuric acid and over, with which they chiefly deal, it is not quite accurate where lower acidities are concerned. For all the amounts of manganese tested (0·0025–0·5 mgrm.) the rate of oxidation appears to be a minimum when the concentration of sulphuric acid is about 9–10 per cent., the colour appearing only very slowly at this concentration. Above this point the rate does increase with increasing acidity, but below it the rate increases as the acidity diminishes. Below 1 per cent. the colour develops very rapidly, but the solutions show the same yellowish tint and tendency to rapid fading as are found when the acidity is too great.

In view of these observations—the possibility of fading if the acidity is greater than 15 per cent. or less than 1 per cent., and the slowness of the reaction at 9–10 per cent., it is recommended that in dealing with animal tissues conditions should be regulated so that the final acidity is about 5–6 per cent.

In the analysis of plant material, which has in general a higher manganese content, it is very seldom *essential* that this low acidity should be maintained, but it is simpler to adopt the same procedure for all classes of material, and to increase the acidity to 15 or 20 per cent. after addition of the periodate, if it is evident that considerable amounts of manganese are present.

It seems probable, in view of our results, that the "unexplained failures" sometimes experienced by Reiman and Minot[5] in using solutions of greater acidity than 2 per cent. nitric acid, have occurred in cases where the manganese present was very small in amount, and the variable acidity in the final solution arising from their acid-sulphate-fusion method of ashing, happened to overstep the limit permissible for traces of manganese.

II. THE REMOVAL OF CHLORIDES.—According to Willard and Greathouse, the presence of chloride does not interfere with the oxidation, as the chlorine may be driven off by adding excess of periodate. This may, however, involve boiling the solution for a considerable time before the colour comes up at all, and if the chlorine is incompletely removed, there is danger of the colour fading to some extent before the estimation is finished. If little manganese is present, this continued boiling is inadvisable, in view of the careful regulation of the acidity which has been found necessary. Hence it is recommended that all chlorides should be destroyed before the oxidation stage, by repeated evaporation with concentrated sulphuric acid—a point on which Wester also lays stress.[6]

DETAILS OF METHOD AS ADOPTED.—I. *Preparation of the Material for Oxidation.*—The process of wet ashing and the acid-sulphate-fusion method described by Reiman and Minot, are both rather cumbersome in view of the large numbers of routine analyses to be carried through, and the necessity for ease in manipulation at all stages of the determination. The method adopted therefore is that of ordinary ashing, with subsequent treatment of the ash with various acids.

Ash the material as completely as possible (in a silica basin) at a low-red heat, and evaporate to dryness with a little concentrated hydrochloric acid. Add a few c.c. of sulphuric acid (33 per cent. by volume), and 3–4 drops of concentrated nitric acid, and evaporate carefully to dryness on a water bath and sand bath, finishing off by gentle ignition over a Bunsen flame. Add 2–2·5 c.c. of sulphuric acid (33 per cent.) and a little water, and evaporate to white-fuming stage, thus removing all traces of chlorides.

After cooling, dilute and filter into a small flask (50 c.c.) for oxidation. Add to the solution one or two small pieces of pumice stone, previously purified by boiling with 5 per cent. sulphuric acid and a little periodate. Evaporate down to about 10 c.c., when the concentration of sulphuric acid will be 5–6 per cent. (allowing for the loss of acid at the fuming stage), and the solution is ready for oxidation.

II. *Oxidation.*—Add 0·3 grm. of sodium periodate (or potassium periodate), and insert a loosely-fitting pear-shaped glass stopper in the neck of the flask. Heat to boiling, and immerse in boiling water for 30 minutes. (Wooden test-tube holders serve to hold the flasks in position in the bath.)

If the whole solution is required for the colour-comparison, cool, and transfer for the determination to the colorimetric tube described below.

If the solution has sufficient colour to require dilution, either (*a*) dilute to the appropriate volume with 5 per cent. sulphuric acid that has been boiled with a little periodate, or (*b*) add water till it has nearly the required dilution, and heat for 15 minutes longer in the bath. Transfer to a calibrated flask, make up to volume, and estimate the manganese by comparing with the standard solution.

III. *Colorimetric Tubes.*—The tubes used are 10 c.c. calibrated cylinders of uniform bore (with a total capacity of 12–13 c.c., and length of 12–13 cm.), graduated

in tenths of a c.c., and made without the usual flange at the bottom, so that the tubes can be held closely together for the colour-comparison. These tubes are most suitable for very dilute solutions. With a standard which contains 0·001 mgrm. of manganese per c.c., 7–10 c.c. give a very convenient depth of tint for matching, but a detectable pink colour can be obtained with as little as 1 c.c., and differences of 0·2 c.c. give distinctly recognisable differences in tint.

If preferred, a colorimeter may be used when the solution is sufficiently strong, but in many cases the amount of manganese in organic materials is so small that it would require an excessive amount of material to give sufficient depth of colour for the colorimeter. Hence we prefer to work always with the above tubes, and usually with the above standard, bringing the unknown solution approximately to this strength by choosing suitable amounts of material for oxidation, and by suitable dilution.

[It may be noted that Wester, in his critical study of the persulphate method, found it necessary to have special colorimetric tubes made with ground-glass caps, to protect the solutions from the action of reducing fumes. With the periodate method, we have found no necessity for such covers, under ordinary laboratory conditions.]

IV. *Standard Solutions.*—(*a*) *Stock Solution of Manganese Sulphate.*—Pure potassium permanganate (0·144 grm.) is dissolved in about 100 c.c. of water, and reduced by sulphur dioxide. Instead of passing in sulphur dioxide, it is much simpler to generate the gas in the solution itself, by means of sulphuric acid and sodium sulphite or bisulphite. The solution is heated till the reaction takes place, and the excess of sulphur dioxide is then boiled off. This method of reduction does not seem to interfere in any way with the stability of the standard solutions. Dilute to 1 litre (1 c.c. of this solution = 0·05 mgrm. of manganese).

(*b*) *Standard Solution for Comparison.*—Oxidise 20 c.c. of stock solution with periodate in the usual way, and make up to 1 litre with 5 per cent. sulphuric acid (previously boiled with periodate). (1 c.c. of this solution = 0·001 mgrm. of manganese). This is the most useful concentration for the colorimetric tubes above described, but stronger solutions may be used if desired.

(*c*) *Stability of Standard Solutions.*—Willard and Greathouse state that a remarkable feature of the solutions oxidised by periodate is their great stability when a slight excess of the reagent is present. Such a solution, kept for 3 months in a stoppered flask, when compared with a similar solution freshly oxidised, showed no change whatever.

We have found that even the very dilute standard described above (0·001 mgrm. of manganese per c.c.) shows the same stability observed by Willard and Greathouse.

V. *Purity of Reagents.*—A great advantage of the procedure outlined is the fact that the only chemicals introduced during the ashing process are the strong

acids—hydrochloric, nitric, and sulphuric—and tests have shown that there is no difficulty in obtaining these entirely free from manganese. Nor have we ever found in our tests that any manganese is introduced as impurity from the silica basins, when subjected to the action of the acids as directed in the estimation. Reiman and Minot, on the other hand, find that the quartz beakers used in their acid-sulphate-fusion method frequently contain manganese, which is dissolved during the fusion process, resulting in contamination, which may amount occasionally to as much as 0·020 mgrm.

The only other chemical introduced in the present method is the periodate, which is almost invariably quite free from manganese. In only one lot have we found a slight manganese impurity, amounting to only 0·0005 mgrm. for 0·3 grm. periodate, the amount used in the determination.

MODIFICATIONS OF USUAL PROCEDURE.—The procedure described above is applicable to the great majority of vegetable and animal tissues, but in a few cases a slight modification of procedure is necessary:—(*a*) For substances containing much calcium, *e.g.* milk; (*b*) for substances containing much iron and very little manganese, *e.g.* blood.

(*a*) *Substances containing much Calcium.*—Unless most of the calcium is removed, it is difficult to avoid loss by spurting in evaporating to dryness with sulphuric acid. There is also danger of the minute amount of manganese present (*e.g.* in milk) being carried down with the calcium sulphate if the ash is treated with sulphuric acid in the usual way. The procedure adopted is as follows:—

Determination of Manganese in Milk.—One hundred c.c. of milk in a silica basin are evaporated down on the water-bath, protecting from possible contamination by particles of rust, which may contain considerable manganese. After ashing completely, and taking down to drynesss with hydrochloric acid, the ash is moistened with a little water, and broken up as finely as possible. Twenty-five c.c. of boiling sulphuric acid (33 per cent. by volume) are added, with careful stirring. After thorough cooling (overnight, if convenient), filter off into another silica basin, washing 5–6 times with 5 c.c. of 33 per cent. sulphuric acid. (N.B. The filter paper must have been previously tested for its resistance to this strength of acid. If the paper available is not strong enough for 33 per cent. acid, 20–25 per cent. may be used, as this will remove sufficient of the calcium sulphate to permit subsequent evaporation to dryness. Ten per cent. acid does not remove the calcium sufficiently.) Evaporate the filtrate to dryness and proceed as usual, except that it may be necessary, when filtering at the final stage before oxidation, to re-filter several times before washing, in order to get an absolutely clear solution.

(*b*) *Substances containing much Iron and very little Manganese.*—If the amount of manganese present is minute, and the ratio of iron to manganese is high, *e.g.* in blood and spleen, proceed thus:—After evaporation to dryness with sulphuric acid, and ignition over a Bunsen burner, instead of adding 2–2·5 c.c. of sulphuric acid (33 per cent.), use a mixture of 1–2 c.c. of syrupy phosphoric acid and 0·5 c.c.

sulphuric acid. The phosphoric acid is used to reduce the yellow tint of the iron salt at the final matching stage. Further procedure is as usual. With no other animal tissue do we find this modification necessary. In the case of liver, the high amount of iron does not interfere, on account of the dilution necessitated by the high manganese content of liver.

TESTS OF METHOD.—The method has been applied in a large number of analyses of plant and animal tissues, with very satisfactory results, judged by (1) concordance of parallel determinations on the same sample, and (2) recovery of added manganese from the samples analysed.

Some results for different classes of material are shown in Tables I and II.

TABLE I.

CONCORDANCE OF PARALLEL DETERMINATIONS.

Substance.		Manganese content of various samples. (Mgrms. of Mn per 100 grms. of dry matter.)	Average. (Mgrms. of Mn per 100 grms. of dry matter.)
Grass		61·3, 61·2, 61·7, 61·2	61·4
Tapioca		0·676, 0·673, 0·698, 0·681	0·682
Potatoes	I.	0·707, 0·705, 0·711, 0·704	0·707
(peeled)	II.	1·19, 1·15, 1·18, 1·18	1·18
	III.	0·467, 0·475, 0·473, 0·475	0·473
	IV.	0·677, 0·638, 0·655, 0·650	0·655
		(Mgrms. of Mn per 100 grms. of moist tissue.)	(Mgrms. of Mn per 100 grms. of moist tissue.)
Liver	I.	0·371, 0·368	0·370
(various	II.	0·229, 0·227	0·228
species)	III.	0·412, 0·420	0·416
	IV.	0·231, 0·237	0·234
	V.	0·248, 0·250	0·249
	VI.	0·137, 0·144, 0·137	0·139
Kidney	I.	0·152, 0·162	0·157
	II.	0·174, 0·174	0·174
Spleen (*b*)		0·018, 0·018	0·018
Milk (*a*)	I.	0·0039, 0·0048, 0·0042, 0·0038 0·0043, 0·0039, 0·0040, 0·0045 0·0044, 0·0039, 0·0040, 0·0040	0·0041
	II.	0·0037, 0·0039, 0·0040, 0·0037 0·0051, 0·0043	0·0041

(*b*) By modification of method for substances containing much Fe, and little Mn.
(*a*) ,, ,, ,, ,, much Ca.

TABLE II.

RECOVERY OF ADDED MANGANESE.

Substance.	Amount analysed. Grms.	Mn added. Mgrms.	Total Mn found. Mgrms.	Manganese in substance taken. Mgrms.	Added Mn found. Mgrm.	Recovery. Per Cent.
Grass	2·011	0·25	1·48	1·23	0·25	100
	2·010	0·25	1·46	1·23	0·23	92
	2·011	0·50	1·76	1·23	0·53	106
	2·011	0·50	1·74	1·23	0·51	102
	2·010	1·00	2·16	1·23	0·93	93
	2·013	1·00	2·20	1·23	0·97	97
Tapioca	10·003	0·025	0·095	0·068	0·027	108
	10·007	0·050	0·122	0·068	0·054	108
	c.c.					
Milk	100	0·0025	0·0067	0·0041	0·0026	104
	,,	0·0025	0·0067	,,	0·0026	104
	,,	0·0050	0·0097	,,	0·0056	112
	,,	0·0050	0·0095	,,	0·0054	108
	,,	0·010	0·0133	,,	0·0092	92
	,,	0·010	0·0136	,,	0·0095	95
	,,	0·020	0·0241	,,	0·0200	100
	,,	0·020	0·0237	,,	0·0196	98
	,,	0·040	0·0440	,,	0·0399	100
	,,	0·040	0·0435	,,	0·0394	99
	34	0·080	0·080	0·0014	0·0786	98
Blood	25	0·0025	0·0039	0·0012	0·0027	108
	,,	0·0050	0·0063	,,	0·0051	102
	,,	0·020	0·0194	,,	0·0182	91
	,,	0·040	0·0397	,,	0·0385	96
	,,	0·080	0·0756	,,	0·0744	93

SUMMARY.—The periodate method of Willard and Greathouse for the colorimetric estimation of manganese can be applied to the estimation of very small amounts of manganese, such as occur in biological material, if due care be taken to drive off all chlorides before oxidation, and to see that the acidity of the solution does not exceed 15 c.c. of sulphuric acid per 100 c.c. of solution. It is recommended for reasons given, that the acidity be kept about 5 to 6 per cent., unless it is found that very considerable amounts of manganese are present. It has been shown that the method gives satisfactory results, both as regards agreement of parallel determinations, and as regards recovery of added manganese from the samples analysed.

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The Determination of Quinine, Cinchonine and Cinchonidine with the Quinhydrone Electrode, and the Choice of End-points in Alkaloidal Titrations.

By E. B. R. PRIDEAUX, M.A., D.Sc., F.I.C., and F. T. WINFIELD, B.Sc.

DIRECTIONS for the titration of alkaloids with acids are often lacking in precision, and this applies particularly to the descriptions of the end-points. Ingenious methods, such as the use of iodeosin with ether, appear to be effective, although they are difficult to explain by the ordinary theories of solutions. Straightforward titrations in aqueous solutions are to be preferred in general, and these are now available except in the case of the very weakest bases. The best conditions can be defined, since the changes in *p*H during neutralisation, including the end-points have been determined colorimetrically (Kolthoff, *Biochem. Z.*, 1928, **662**, 289), and also, in the case of those which resist reduction, such as pyridine and piperidine, by the hydrogen electrode (Muller, *Z. f. Electrochem.*, 1924, **30**, 587; Prideaux and Gilbert, *J. Chem. Soc.*, 1927, 2164). From these results, the dissociation constants are calculated in the usual manner, and the values of *p*H at the end-point, *e.g.* *B*.HCl, are deduced. This point may conveniently be called the titration exponent *p*T; which is the same term as that which is applied to indicators to express the *p*H at which the colour change is most marked to the eye. Indicators should then be chosen rationally so that their *p*T values are as near as possible to the *p*T's of the base hydrochlorides, &c. In any case, this *p*T must be included in the indicator range.

GROUPING OF BASES.—From this point of view, bases fall into a few groups, connected by transitional members.

(*a*) Strong monacid bases $k=1\times10^{-3}$ to 1×10^{-4}. The aqueous solutions of the salts react neutral or very slightly acid, and any ordinary indicator changing at $pH=7{\cdot}0$ to $5{\cdot}0$ may be used. Examples are: piperidine, coniine, berberine, hydrastinine.

(*b*) Weaker monacid bases having constants $k=1\times10^{-5}$ to 1×10^{-6}. The higher value is nearly equal to the constant of ammonia, and therefore methyl red is the ideal indicator for these titrations. Among alkaloids which can be titrated accurately thus are: atropine $pT=5{\cdot}7$, cocaine 5·05, tropacocaine 5·8. Monacid bases having $k=1\times10^{-6}$ will give *p*T values from 4 to 5 according to their concentrations, as defined by the equation—

$$pT=pk_w/2-pk_B/2-\tfrac{1}{2}\log c. \quad *$$

* k_W is the water constant ($=1\times10^{-14}$), k_B is the dissociation constant of the base, k_1 and k_2 refer to the two constants of a diacid base. The symbol '*p*' denotes the negative exponent, or negative value of $\log_{10}$.

These can still be titrated with methyl red, methyl orange, bromphenol blue and bromcresol blue, but the total concentration should be known, and a comparison made with a finished titration on the appropriate buffer solution containing the same amount of indicator. Bases of this class are rare—an example is codeine.

An important class is that of the diacid alkaloids having k_1=about 1×10^{-6} and $k_2=1\times10^{-8}$ or less. Their titration exponents (to *B*.HCl) are calculated in an entirely different manner, *i.e.* from the equation—

$$(OH') = \sqrt{\frac{k_1 k_2 \times c}{k_1 + c}}.\ \dagger$$

At all ordinary concentrations the first constant can be neglected in comparison with c, and the equation reduces to—

$$(OH') = \sqrt{k_1 k_2} \text{ or } pH = pk_w - \frac{pk_1 + pk_2}{2}$$

("*p*" stands for the negative logarithm throughout). One important difference between this and the equation for a monacid base, is that *p*H at *B*.HCl=*p*T is now practically independent of the total concentration. Another is, that, quite a low value of k_1, *e.g.* 1×10^{-6}, when associated with not too low a value of k_2, *e.g.* $>1\times10^{-12}$, gives a *p*T of about 5, which is not too far removed from neutrality and gives a good titration.

To this class belong nicotine, the cinchona alkaloids, brucine and strychnine. The values of *p*T, tabulated below, evidently do not vary greatly with the concentration. Since they are little, if at all, below 6·0, methyl red is not the appropriate indicator, for it will be nearly, or completely, yellow at the end-point. Bromcresol purple is suitable, and *p*-nitrophenol has also been recommended (for quinine). If it is necessary to titrate in alcoholic solution (50 per cent.), bromphenol blue has been found to give good results, in the titration of brucine and strychnine.

USE OF THE QUINHYDRONE ELECTRODE.—The quinhydrone electrode offers many advantages. According to the usual specifications, it consists of a platinum wire electrode dipping in the solution saturated with quinhydrone and connected by a salt-bridge with a standard electrode or half cell, such as the decinormal, normal or saturated KCl calomel electrode. The potentials E_0 of the first and third of these electrodes against platinum quinhydrone in solutions of normal

† The value of [OH'] or hydroxyl ion concentration at the first equivalence point of a diacid base is given by the equation—

$$(OH') = \sqrt{\frac{k_1 k_2 \times c}{k_1 + c}}$$

When k_1=about 1×10^{-6} and the concentration 'c' is not too low, this reduces to the expression given. Thus in the case of quinine, etc., which have in round numbers—$k_1=1\times10^{-6}$, $k_2=1\times10^{-10}$, the solutions of these equations give—

$$(OH') = \sqrt{1\times10^{-6}\times1\times10^{-10}} = 1\times10^{-8}$$

$$\text{or } pH = 14 - \frac{6+10}{2} = 6$$

hydrogen ion concentration are 0·366 and 0·453 at 18° C. (quinhydrone positive and diffusion potentials eliminated by the salt-bridges). When (H·) in the solution to be measured is as usual, less than 1·0 N, then its value (as pH) is given by—

$$E = E_0 - 0{\cdot}058\, pH.$$

Thus, if the potential of a certain quinhydrone electrode against saturated KCl calomel is 0·350 volts, we may calculate pH and (H·).

$$pH = \frac{0{\cdot}453 - 0{\cdot}350}{0{\cdot}058} = 1{\cdot}78,\ (H^{\cdot}) = 1{\cdot}66 \times 10^{-2}.$$

Since the quinhydrone electrode cannot be used in alkaline solutions (beyond pH = 8), the titration is best carried out by dissolving the base in a known excess of standard acid and titrating back with alkali. The results with very weak bases, or with salts of any bases at high dilutions, are certainly more accurate than those which can be obtained with any indicators. For although the pH, x curves (x = degree of neutralisation of base with HCl, deduced from amount of alkali added) are too flat even at the point BHCl to give sharp colour changes, yet the maximum ΔpH/Δx obtained potentiometrically is sufficiently definite, or, at any rate, "x" corresponding to the correct pT, can be found.

The tabulated results represent actual titrations in solutions of various concentrations at ordinary temperatures. The values of $\Delta p/\Delta$x have been expressed on a uniform scheme in which x = fraction of an equivalent of alkali or acid added to equivalent of the base, present as salt.

Quinine (0·1 *molar*) gave a precipitate before the end-point BHCl was reached.

QUININE (0·05 *molar*).—The titration was carried out by adding standard acid to a solution of the mono-hydrochloride, BHCl.

pH =	6·22		5·65		5·38		5·22		4·78
ΔpH/Δx =		<u>17·07</u>		8·04		4·89		2·95 §	

The backward titration was carried out by adding alkali to the base dissolved in excess of standard acid.

pH =	5·38		5·48		5·61		5·80		6·10		6·20		6·30
ΔpH/Δx =		5·0		6·5		9·5		15·0		<u>20·0</u>		14·3	

The maxima are underlined in each case. In the back titration, the end-point came at about 1 per cent. short of the theoretical amount of alkali.

0·01 *molar*.—A quantity equivalent to 0·05 molar solution of the base was dissolved in 0·10 N hydrochloric acid. Twenty ml. of this with 80 ml. of water were titrated with 0·1085 N alkali.

pH =	5·26		5·80		6·29		6·55		6·76		6·93
ΔpH/Δx =		10·0		22·5		<u>24·0</u>		19·5		16·0	

§ ΔpH/Δx, the rate of change of pH with added titrant, reaches a maximum at the equivalence point. The values of these maxima in 0.01 molar solutions of quinine salt were calculated from the experimental results of Baggesgaard-Rasmussen and Schon.

(calculated from the results of Baggesgaard-Rasmussen and Schon, *Z. Elektrochem.*, 1925, **81**, 189).

0·005 *molar.*—The base, dissolved in excess of acid, was titrated back with standard alkali.

pH	= 5·04		5·25		5·60		5·98		6·39		6·87		7·12	
$\Delta pH/\Delta x$	=	8·15		17·3		19·5		21·9		11·4		8·6		

0·002 *molar.*—The base (0·06488 grm.) dissolved in 4·968 grm. HCl (from weight burette) which is 0·410 grm. in excess of that required for *B*HCl was made up to 100 ml. and titrated back with 0·0795 *N* NaOH from a micro burette. x is the degree of formation of the salt BH_2Cl_2.

x	= 0·108		0·059		0·0455		0·007		−0·025		−0·086
pH	= 5·294		5·662		5·810		6·216		6·572		7·033
$\Delta pH/\Delta x$	=	7·5		10·9		10·6		11·1		7·5	

Even in this dilute solution, the end-point is defined to ±0·015 in x, *i.e.* with an accuracy of 1·5 per cent., and could be defined more closely still by more gradual addition of alkali. This titration was done at 10° C. in a thermostat. The value of pT is 6·3, while those in the more concentrated solutions were 6·1 to 6·2.

CINCHONINE.—The titration of this base in 0·1 *M* solution followed exactly the same course as that of quinine, and precipitation again occurred before the end-point. The values of Baggesgaard-Rassmussen and Schon in 0·01 molar solutions also show no material difference from those of quinine.

0·002 *molar.*—The base (0·0588 grm.) was dissolved in 5·151 grm. of hydrochloric acid, which is 0·595 grm. in excess of *B*.HCl, and treated as described under "quinine" at 18° C.

x	= 0·200	0·133	0·097	0·029	+0·011	−0·002	−0·018	−0·036	−0·054
pH	= 4·856	5·083	5·231	5·766	6·012	6·224	6·545	6·700	6·845
$\Delta pH/\Delta x$	=	4·11	7·87	13·66	23·6	20·1	8·55		

The end-point is defined to ±0·005 in x, *i.e.* with an accuracy of 0·5 per cent. The alkali was added more gradually than in the case of quinine, but the additional accuracy is also due to the higher values of $\Delta pH/\Delta x$. In spite of the identity of both constants, the titration of cinchonine is sharper than that of quinine, as will be seen on plotting graphs of the above results. The value of pT is slightly lower, *i.e.* 6·20.

CINCHONIDINE. 0·100 *molar.*—This base, stereoisomeric with quinine, resembles it very closely in its neutralisation equilibria, but differences become apparent in the most dilute solutions.

A weight of the base equivalent to 25 ml. of 0·1 *N* acid was dissolved in 25 ml. of 0·2 *N* acid (factor 1·037) and was titrated back with 0·1 *N* alkali (factor 0·898).

x	=	0·060		+0·025		+0·011		+0·004		−0·004
pH	=	5·455		5·92		6·29		6·52		6·77
$\Delta pH/\Delta x$	=		10·4		26·3		32·8		31·3	

The end-point is probably at +0·004, and is defined with an accuracy of 0·2 per cent. The value of pT is 6·4.

Cinchonidine 0·002 *molar*.—0·0588 grm. dissolved in 4·960 grm. of HCl, which is 0·404 grm. in excess of that required for *B*.HCl was treated as described under "quinine" at 18°.

x	= 0·202	0·134	0·051	0·026	+0·001	−0·030	−0·048	−0·083
pH	= 4·644	4·837	5·259	5·390	5·750	6·276	6·504	6·722
$\Delta pH/\Delta x$	=		5·24	14·44	17·0	12·7	6·23	

The end-point is defined to 0·015 in x, *i.e.* with an accuracy of 1·5 per cent., which could be increased by more gradual addition of alkali. The value of pT is 5·8, which is much lower, *i.e,* more acid than the end-points of quinine and cinchonine. Consequently, it should be possible to titrate this alkaloid in dilute solution with methyl red.

We have tested this conclusion by a potentiometric titration in the presence of this indicator, and have found that a full yellow gives theoretical results.

General Conclusions with regard to Indicators.—The titration exponents of all three alkaloids in 0·10 to about 0·05 molar solutions lie within the range 6·2 to 6·4. The choice of the indicators p-nitrophenol (5·0–7·0) and bromcresol purple (5·2–6·8) is therefore justified. In the case of 0·10 to 0·05 molar quinine and cinchonidine, bromthymol blue (6·0–7·6) should also be available. Dilute cinchonine, 0·002 molar, should be titratable with the use of ethyl red (4·5–6·5), and dilute cinchonidine, 0·002 molar, with ethyl red, as well as methyl red (4·2–6·3).

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Official Appointments.

The Minister of Health has confirmed the following appointments:—

A. J. C. Lickorish, F.I.C., as Public Analyst for the City of London (April 24th, 1930).

Herbert John Evans, F.I.C., as Public Analyst for the County Borough of St. Helens (June 14th, 1930).

Harold F. Barke, F.I.C., as Public Analyst for the Borough of New Sarum (Salisbury City) (August 1st, 1930).

W. H. Roberts, MSc., F.I.C., as Public Analyst for the County Borough of Southport (August 14th, 1930).

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

ERIC VOELCKER, F.I.C., A.R.C.S., as Deputy Agricultural Analyst for Berkshire, Buckinghamshire, Oxfordshire, the East Riding of Yorkshire, and the Isle of Ely, E. W. Voelcker having resigned (July 26th, 1930).

S. EMSLEY, B.Sc., F.I.C., as Agricultural Analyst for the Isle of Wight (July 26th, 1930).

W. H. ROBERTS, M.Sc., F.I.C., as Agricultural Analyst for the County Borough of Birkenhead (July 26th, 1930).

HERBERT JOHN EVANS, B.Sc., F.I.C., as Agricultural Analyst for the County Borough of St. Helens (July 26th, 1930).

F. MAUDSLEY, B.Sc., F.I.C., as Agricultural Analyst for the County Borough of Burnley (July 26th, 1930).

RICHARD WILLIAM SUTTON, B.Sc., F.I.C., as Deputy Agricultural Analyst for Leeds (July 26th, 1930).

C. A. SEYLER, B.Sc., F.I.C., as Agricultural Analyst for Swansea (July 26th, 1930).

A. J. C. LICKORISH, F.I.C., as Agricultural Analyst for the City of London and Port of London (July 26th, 1930).

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.

PRESENCE OF BLUE MINERAL COLOURING MATTER IN "PURE" GELATIN.

IN the routine testing of a pharmaceutical preparation containing a small percentage of gelatin, a marked deposit of insoluble material, deep blue in colour, was observed in several batches. Examination of the various raw materials used showed that the contamination came from the gelatin.

The gelatin had been purchased in the ordinary way and sold as "Gelatin B.P." When a warm 20 per cent. solution was centrifuged, a marked deposit of a deep blue dye was again observed. This dye showed the characteristic reaction of ultramarine when tested with strong hydrochloric acid.

Enquiries were made through the intermediate supplier of this sample of gelatin, who was informed by its manufacturer that the practice of adding ultramarine to gelatin was quite normal.

It should be added that the manufacturer of this gelatin was not English; a subsequent supply of gelatin B.P. from a manufacturer in this country has been found to be entirely free of any traces of insoluble dyestuff.

A. L. BACHARACH.
G. N. GRINLING.

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GELATIN IN CREAM.

A VENDOR was prosecuted in Melbourne on a charge that he sold cream which had been thickened by the addition of 0·4 per cent. of gelatin. The second sealed sample was examined for the defence in this Laboratory, in accordance with the provisions of the Health Act. Stokes's test was applied by adding acid mercuric nitrate solution, filtering, and adding cold saturated picric acid solution. A copious precipitate was obtained, distinguished from a "sour cream" precipitate by its solubility in hot water. The filtrate from the acid mercuric nitrate precipitate also gave the same reactions as a gelatin solution; with alcohol (positive), tannic acid (positive), and formaldehyde (negative). A positive test was therefore reported. The defendant strongly denied that gelatin had been employed, but stated that he had added rennet to the cream after pasteurisation, for the purpose of thickening it. Further tests were therefore made. Rennet liquor and cream of known purity were tested separately as described, and neither gave a precipitate. They were then mixed and re-tested, and again gave no precipitate. After standing at room temperature for 24 hours, the mixture gave a strongly positive result, *viz.* a copious precipitate.

It is therefore evident that Stokes's test cannot be accepted as conclusive for the presence of gelatin in cream, since the same reaction is given by degradation products of milk proteins. It is suggested that the precipitate should be further subjected to Hopkins's test for tryptophane, since this amino-acid is not present in gelatin.

O. A. MENDELSOHN.

MELBOURNE, AUSTRALIA.

THE DETECTION AND DETERMINATION OF SESAME OIL WHEN MIXED WITH OTHER EDIBLE OILS, WITH PARTICULAR REFERENCE TO ARACHIS OIL.

THE following modification of Baudouin's test has been used with success in this laboratory:—

Five grms. of the oil are introduced into a separator together with 5 c.c. of concentrated hydrochloric acid. Four drops of a 1 per cent. alcoholic solution of furfuraldehyde are then added, and the resulting mixture well shaken for 2 minutes and allowed to stand for 3 minutes.

The acid layer is run off into a 50 c.c. Nessler glass, and a further quantity of concentrated hydrochloric acid is passed through the separator to wash out the last few drops of the acid layer. The mixture is then made up to the mark with concentrated hydrochloric acid, and the red colour so produced compared with known standards treated in precisely the same way. The solutions are not always quite clear, but it has been proved that this does not introduce any inaccuracy.

It has been found possible to judge easily, to the nearest 5 per cent., up to 40 per cent. of sesame oil in a given mixture. Where a greater percentage of sesame oil is present the test does not appear to be quite so sensitive.

It is usually necessary to make the standards simultaneously with the test solution, as the colour deteriorates on standing. The rate of deterioration is not great enough to affect the validity of the result if the work is carried out with reasonable speed.

Dilute hydrochloric acid was tried in place of concentrated hydrochloric acid as the diluent, but the colour is less intense and deteriorates much more rapidly under these conditions. However, the concentrated hydrochloric solution is easily mixed by pouring it into a second Nessler glass.

When examining mixtures of sesame and arachis oils Bellier's test is carried out in addition. The percentage of sesame oil, deduced from the percentage of arachis oil present, is usually found to be in close agreement with the result obtained colorimetrically. Both oils are very extensively used for domestic purposes in Burma, and there is a temptation to adulterate the more expensive sesame oil, with the less expensive arachis oil in all proportions.

The oils are also apt to be adulterated with mineral oil. In this case the above tests have been found useful when taken into consideration with the determination of the unsaponifiable matter.

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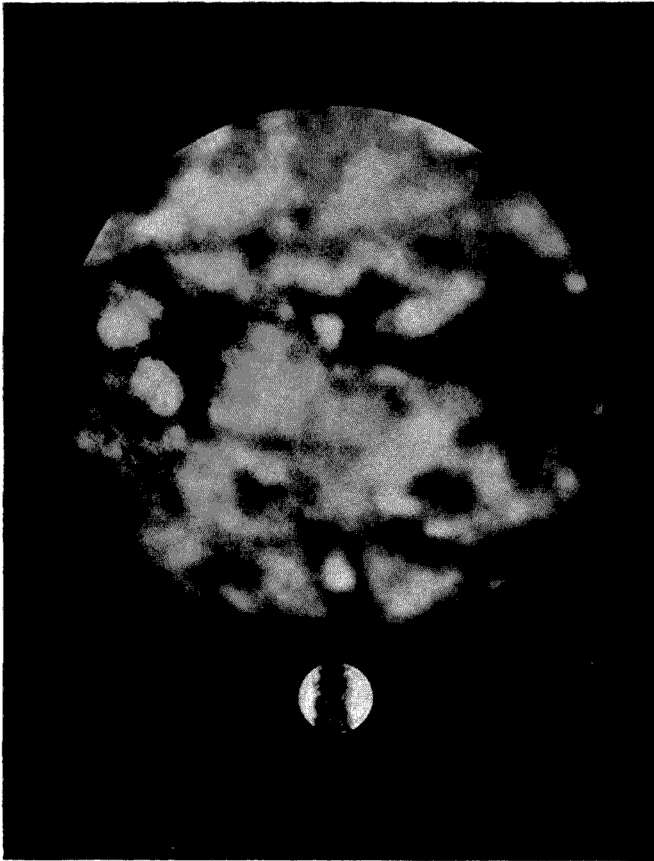
THE PHOTOMICROGRAPHY OF INK SEDIMENTS IN PEN WRITING.

THE sediment gradually accumulating in an ink-pot may consist of substances thrown out of solution from the ink or extraneous materials, such as plant débris, pollen, starch grains, insect fragments, wool fibres, etc., and such sediment often forms part of the written characters. This insoluble matter in the handwriting, owing to its characteristic structure and arrangement as observed under the microscope, may be of considerable value in identifying ink derived from a particular receptacle or in indicating the common source of the writing on two or more different documents. The sediment is naturally more conspicuous in portions of the writing in which the ink pigment is relatively small in amount, such as portions blotted while wet or where fading has occurred, but, although ocular microscopic examination may enable the structure of the sediment to be seen, photomicrography with the usual liquid or gelatin colour filters seldom yields satisfactory results. This difficulty, however, may often be overcome by the use of a filter consisting of dilute blue-black ink, and although the best definition is not obtained by this means, the results enable a ready comparison of different sediments to be made. The procedure adopted for the example illustrated was as follows:—The source of illumination was a 200 c.p. half-watt electric lamp with condenser, the beam of light passing through a glass cell containing a 10 mm. layer of blue-black ink diluted with 40 volumes of water. With this illumination and a magnification of $\times 110$ an exposure of 20 minutes was required with an Imperial Process plate. The small photomicrograph below shows a portion of a "t" on a document $\times 7$, whilst the larger one represents part of the same original under a magnification of 110 diameters. The sediment is shown in the form of amorphous blotches and streaks and is in striking contrast to many other deposits met with. A similar application of coloured inks as filters would probably be of equal utility in the investigation of handwriting in coloured inks.

T. J. WARD.

Erratum.—A MODIFIED GUTZEIT APPARATUS. The Note on pp. 503-504 of the August issue, should have been signed "A. J. Lindsey" (not "Linsey").



PHOTOMICROGRAPH OF INK DEPOSIT IN PART OF A LETTER **t** ON A DOCUMENT.

Below magnified × 7.
Above " × 110.

Notes from the Reports of Public Analysts.

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

CITY OF BIRMINGHAM.

REPORT OF THE CITY ANALYST FOR THE SECOND QUARTER, 1930.

DURING the quarter 1252 samples were analysed, of which 59 were formal and 1193 informal samples. Forty-five of the total samples were incorrect.

NITRATES IN MILK.—Two formal samples and a previous informal sample coming from one farm contained traces of nitrates. On a visit to the farm it was found that the empty churns on the premises each contained a small quantity of water, which was said to prevent their rusting and to keep them clean. It is quite possible that this procedure was the cause of the presence of added water in the milk.

Nitrates were found in four other samples from another farm, and, on a visit to the farm, it was found that the samples then taken were of very good quality and free from nitrates. A sample of the water taken at the farm contained a large percentage of nitrates.

LABELLING OF MARGARINE.—Inside the wrapper of a sample was a printed slip bearing the words: "—— rolls. Contain 6 per cent. of butter." According to Section 6 (d) of the Food and Drugs Adulteration Act, margarine must not be described on any label by any name other than either "Margarine," or a name combining the word "Margarine" with a fancy or descriptive name approved by the Minister of Agriculture. In a communication from the Ministry it was stated that the name "——" had been authorised, but not the remainder of the wording relating to butter. In a case taken to the High Court in 1923, it was held that the description "*Mayco* margarine mixed with 'Maypole' butter" was a contravention of the section, it being held that the words "mixed with Maypole butter" were an integral part of the name. In 1925 another decision of the High Court held that the words "contains a small quantity of butter" included in the description of a margarine were merely descriptive and not part of the fancy or descriptive name, and that therefore no offence was committed, the previous decision of 1923 being doubted. In the opinion of the Town Clerk's Department the wording of the present label did not constitute an offence, and no further action was taken in the matter.

WOOD VINEGAR SOLD AS TABLE VINEGAR.—The label on the sample bore in large type the words "Table Vinegar," and in very small type, "sold as Wood Vinegar." It has been held in several cases in Birmingham and other places that "table vinegar" should consist of malt vinegar. The sample actually consisted of artificial vinegar. This label was therefore a contradiction in terms, apart from the intended deception of the small type used. The packers were communicated with and asked either to describe the product simply as artificial vinegar, or, if it were desired to retain the use of the term "Table Vinegar," to supply malt vinegar. The firm decided on the latter alternative, and a sample taken a short time afterwards complied with our requirements.

Teas Sold as Free from Tannin.—On the label of one sample appeared the words "remarkably free from the objectionable tannic acid, the great cause of indigestion to users of ordinary full leaf teas." The actual percentage of tannin in the sample was 13·8 per cent., which is an average figure for this type of tea. The label was therefore a false one. The packers were asked to omit all reference to the freedom from tannic acid on future labels, and agreed to do so.

The label on another sample stated that the tea "is free from crude tannin and practically tanninless." The percentage of tannin in this tea was 14 per cent., and a similar request was made to the packers, which has been complied with.

A third sample bore a label with the words "fine tea contains very little tannin and consequently . . . this tea can be used freely . . . by persons who suffer from indigestion, etc." The percentage of tannin was 12·7 per cent. The packers were approached as in the other cases, with a similar request, and the label will be altered in future.

"Rich Dairy" Cream.—The label of this sample stated that it was "A highly concentrated rich dairy cream." "Butter fat 23 per cent." The actual percentage of fat was 23 per cent. A rich dairy cream, however, should contain at least 50 per cent. of fat, so that the label was false in this particular. The packers agreed to print new labels on which the offending statement should not appear.

Zinc Ointment.—A sample contained only 13·5 per cent. of zinc oxide, instead of 15 per cent. as required by the British Pharmacopoeia. The packers, when communicated with, suggested that the deficiency was due to the zinc oxide settling by gravity, owing to hot weather. There is a possibility that the necessary stirring was not continued until the mixture was cold, as should have been done, and that, on this account, the oxide had partly settled. The retailer, however, is now buying in bulk and filling his own tins. A sample of the ointment taken later was correct.

Compound Calamine Ointment.—There is no definite standard for this ointment, but the sample was very badly dispensed, about 5 per cent. of the base being distributed in large lumps, instead of being properly melted and mixed. There is no excuse for such carelessness, and the vendors were cautioned by the Medical Officer of Health.

Compound Bismuth Lozenges.—This sample did not consist of the British Pharmacopoeia lozenge at all, but of an unofficial lozenge containing only a third of the proper amount of bismuth carbonate, no calcium carbonate, and a considerable excess of magnesium carbonate. It also contained talc, which should not be present at all. The vendors—a small drug store—were cautioned by the Medical Officer of Health.

Extra Strong Seidlitz Powder.—Extra strong Seidlitz powders should either conform to the B.P.C. formula (*i.e.* the amount of Rochelle salt in the blue paper should be half as much again as in the ordinary B.P. powder, the amount of bicarbonate and tartaric acid remaining the same) or else the amounts both of the bicarbonate and the tartaric acid should be half as much again, the Rochelle salt being preferably a little less than in the B.P. powder. In both these cases the mixture resulting from admixture with water would be practically neutral, as a Seidlitz powder should be.

In the sample in question, the bicarbonate was higher than it should have been and the tartaric acid was deficient. The resulting mixture would be highly alkaline. The powder was lemon flavoured and sweetened with saccharine. The packers were cautioned.

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.

OIL OF CASSIA: ARBITRATION DISPUTE AS TO AN ANALYSIS.

NAUMANN *v.* RADERMACHER.

ON March 19th and 20th an application was made to the King's Bench Divisional Court (Lords Justices Greer and Romer) to set aside the award of an arbitrator in a dispute between buyers and sellers.

Mr. Clement Davies, K.C., for the buyers, said that they had bought 10 cases of oil of cassia, and when the consignment arrived they had sent a sample to a well-known analyst. He had reported that it contained artificial cinnamic aldehyde, and was therefore not a normal, natural oil. This was confirmed by the analysis of another chemist, and, as a result, the buyers claimed their right to reject the consignment, and an arbitration followed.

The arbitrator asked for another sample, and sent this to a third analyst, who reported that the cassia oil had a low specific gravity, but that he could not detect any chlorine or any trace of artificial cinnamic acid; he was of opinion that the oil was of merchantable quality. Thereupon the arbitrator, said Mr. Davies, issued his report on the third analysis, without making up his own mind on the subject of quality.

Lord Justice Greer, in giving judgment, said that business men were often satisfied to have their disputes settled upon principles as to the admissibility of evidence which would not be accepted in a Court of Law, and injustice frequently resulted. The parties in this case had agreed that any disputes with regard to the contract should be settled in London "in the usual way." There was evidence that the usual course of procedure had been followed, and even had there been no agreement that the arbitration should be conducted in the way it was conducted, the buyers had put themselves in a position in which they could not complain of what happened, because they had presented their case in a way in which it would not have been proper to present it in a Court of Justice. The motion would be dismissed.

Lord Justice Romer concurred.

On July 16 the buyers appealed against this judgment.

Mr. Clement Davies, K.C., for the appellant, said that the arbitrator in this case had ignored the evidence and had called in the umpire on his own account. A tribunal was not entitled to behave without some regard for the ordinary methods of justice, and he (counsel) submitted that the umpire had acted contrary to natural justice.

Lord Justice Scrutton, in giving judgment, said that fifty years ago the appellant might have met with sympathy from the Courts. But during the last thirty or forty years the attitude of the Courts towards commercial arbitration had considerably changed. Instead of calling witnesses to give evidence before a

judge who, though completely impartial, was also completely ignorant of the subject-matter, business men had arrived at a system by which each side appointed an arbitrator who was a negotiating advocate, and in case of disagreement between them the two appointed an umpire.

In the present case the evidence was overwhelming that the usual way of conducting an arbitration in London was for the umpire to take a sample and have his own independent analysis, and not to have a further meeting nor to submit the evidence to either of the parties unless specially asked to do so. In this case the buyer's arbitrator saw the umpire and submitted two analyses. He admitted that he was told by the umpire that a sample would be wanted and he raised no objection. After that it was not possible for the buyer to object, and it was idle to suggest that he did not know what the sample was for. The umpire got the independent analysis and acted on it without a further meeting of the arbitrators. There was nothing in the procedure to lead the Courts to say that it was contrary to public policy, and therefore that the usual contract for "arbitration in London in the usual way" would not be enforced. The appeal would be dismissed with costs.

Lords Justices Lawrence and Slesser concurred.

DRIPPING WITH EXCESS OF FREE FATTY ACIDS.

On July 11, a Bethnal Green tradesman was summoned at Old Street for selling dripping which contained 2 per cent. excess of free fatty acids; a wholesale bacon company was summoned for delivering dripping which contained 4 per cent. excess free fatty acids; and another Bethnal Green tradesman was summoned for selling dripping which contained 2·6 per cent. of free fatty acids.

Mr. W. Framton, who prosecuted for the Bethnal Green Borough Council, said that the cases were concerned with the sale of dripping in which decomposition of glycerides had occurred, with the result that free fatty acids, which were injurious to health, were produced.

Mr. A. E. Parkes, F.I.C., Public Analyst for Bethnal Green, said that excess of free fatty acids might be injurious, and, in his opinion, the amounts found in the three samples would be prejudicial to the purchaser. In cross-examination, the witness said the prejudice to the purchaser consisted in the fact that the excessive amount of free fatty acids indicated a considerable amount of decomposition. This would be injurious to children and to some people with delicate digestive organs. A perfectly sweet dripping could develop free fatty acids under certain conditions. When he spoke of adulteration he did not suggest that anything had been put into the dripping.

Mr. Ricketts, for the defence, said that the difficulty was that there was no standard for dripping. It was possible for a perfectly good article, prepared from perfectly sound materials, to become, soon afterwards, as these samples were through exposure to the air. His clients were primarily a bacon-curing company, and fat which was rendered down and sold as dripping was one of their by-products. Immediately they heard that there was any complaint about the dripping they had stopped the sale altogether.

The Stipendiary (Mr. Snell) dismissed the summonses against the two retailers, and imposed a fine of £5, with six guineas costs, upon the bacon company.

Ministry of Health.

DETERMINATION OF SUCROSE, LACTOSE AND INVERT SUGAR IN SWEETENED CONDENSED MILK.*

As has been the case with earlier reports in this series from the pen of Dr. Monier-Williams, we have here a critical review of the subject, a study of the literature, and much original work, together culminating in a notable advance. The hope expressed in a prefatory note, to which further reference is made below, that the Report "will be of assistance to Public Analysts and others concerned with the examination of this product" will certainly be realised.

The determination of the sugars is considered in its relation to the Public Health (Condensed Milk) Regulations, 1923. It is regrettable that circumstances have been such that, within the space of four months, there should be, for the determination of sucrose, one method recommended by the Society, and another issued by the Minister of Health. This report, however, goes further, all three sugars, and their reactions upon each other, being considered together.

The view of the Milk Products Sub-Committee of the Society that, for the purposes of the Regulations, the percentage of total milk solids should be ascertained by the subtraction of sucrose (and invert sugar if present) from the total solids, is adopted. For sucrose, a polarimetric method is worked out, but this "is not put forward as preferable to that recommended by the Committee of the Society of Public Analysts, but as an alternative which may be of value in certain circumstances."

As Dr. Monier-Williams points out, one of the main difficulties in the polarimetry of condensed milk is the allowance for the volume occupied by the clarification precipitate. To overcome this, he adopts the principle of Sachs, using dialysed iron as precipitant, washing the precipitate free from sugar, and suspending the washed precipitate in a solution containing known amounts of the sugars (approximately equal to those derived from the condensed milk) in the same volume and filtering again. The percentage of sucrose is then given simply by ratio of the changes in rotations produced on inversion of the two filtrates.

By this means any dependence upon a calculation for the volume of precipitate is avoided; it still has to be assumed, however, that no alteration in volume results from prolonged washing with a boiling 1 per cent. solution of sodium chloride.

The process would seem to be lengthy: the number of polarimetric readings, as compared with one in which the volume correction is calculated from the fat and protein, is at least doubled, further polarimetric readings being necessitated by examination of the washings for freedom from sugar. It is true that the method does not require a knowledge of the percentages of fat and protein, but in any ordinary analysis the fat will have to be determined, and in any critical analysis so will the protein.

Analyses of 18 milk-sucrose mixtures of known composition are given; with a few exceptions, these show a difference of not more than $\pm 0{\cdot}2$ per cent. from the actual when referred to a condensed milk of normal composition. These differences are rather higher than those reported by the Committee previously referred to.

* Reports on Public Health and Medical Subjects, No. 57. Ministry of Health. By G. W. Monier-Williams, O.B.E., M.C., M.A., Ph.D., F.I.C. 1930. Pp. 23. H.M. Stationery Office. Price 6d. net.

The error is attributable mainly to the limit of accuracy of the polarimetric readings (about 0·01° angular). Dr. Monier-Williams considers that it is doubtful whether, using a sodium source of light, which is not perfectly monochromatic, greater accuracy would be obtained by using more concentrated filtrates, or a longer observation tube. The dialysed iron filtrate is neutral—a very favourable point—and is stated to be free from protein. The questions of salt effects, temperature and muta-rotation are discussed.

From the same polarimetric readings the lactose can be calculated, a pleasantly simple method of calculation being devised.

The effect of heating upon the specific rotation of lactose is discussed, and experiments upon this point are recorded: this effect has an important bearing upon the possibilities attaching to various methods of determining invert sugar, a question which occupies the later portions of the Report.

The Report proceeds to consider the determination of the sugars by copper reduction. The dialysed iron filtrates are again used, suitably decalcified. Certain errors inherent in copper reductions are minimised by the procedure adopted: firstly, by determining the reducing powers of the condensed milk filtrate and the filtrate containing known amounts of the sugars, at the same time, in the same bath, identical conditions are attained; then, by carrying out the reductions in 100 c.c. centrifuge tubes, loosely stoppered, back oxidation of the cuprous oxide is minimised; further, in the case of lactose, the use of tables is avoided altogether, the reducing power being linear over the small range involved, and the percentages of lactose being simply proportional to the copper reduced by the two filtrates; in the case of the sucrose, the departure from linearity, unless the proportions of sucrose happen to be identical, necessitates the use of tables, Woy's being selected as being the correct ones under the conditions prevailing. The cuprous oxide is separated and washed in a centrifuge, and is determined by permanganate titration according to the Bertrand procedure, but other recognised methods of determining the reduced copper would be permissible, provided that the two determinations are carried out under strictly comparable conditions. The advice that "if any other of the recognised methods of determining copper reduction be used, the tables appropriate to such method should be used instead of Woy's" should, in the writer's opinion, be acted upon with caution; it is precisely the appropriateness of such tables in the case of condensed milk that is the point at issue, even though the two solutions being compared are approximately of the same composition.

On fresh milk, the differences between the percentages of lactose hydrate, as determined by polarimeter and by the copper reduction method, were from —0·05 to +0·06. An example of a known mixture of milk and sucrose shows excellent agreement between the determined and the actual percentages of sucrose, and the agreement between the polarimetric and the copper reduction determinations on a condensed milk is also satisfactory; but, to quote the report, the copper reduction method "would appear to be as accurate as the polarimetric method, but it has yet to be tested on a larger number of samples."

The procedure devised does, however, put the copper reduction methods on a much more satisfactory basis than has hitherto been the case.

In a report abounding in matters of importance and interest perhaps the most important part is that devoted to the detection and determination of invert sugar. Were it not for the possible disturbance of the specific rotation of lactose—certain in the case of unsweetened, and possible in the case of sweetened condensed milk—the invert sugar could be deduced from a consideration of the polarimetric and copper reduction figures. Dr. Monier-Williams develops the use of Barfoed's

solution for the determination. By submitting the dialysed iron condensed milk filtrate, along with a control fresh milk filtrate containing similar proportions of sucrose and lactose, to the action of Barfoed's solution, under identical conditions a "difference figure" is obtained which represents the reduction due to invert sugar. The use of the control is necessary owing to the slight reduction by lactose and sucrose. This line of attack is highly promising and should prove of great service.

A positive result with the modified Barfoed's procedure as described does not necessarily prove the presence of invert sugar, but only of a monose sugar, but Dr. Monier-Williams considers that if the amount of apparent invert sugar calculated from Barfoed agrees closely with that calculated from the polarimeter and copper results, it is fairly conclusive evidence of invert sugar.

It has been generally assumed that invert sugar is not present in appreciable amounts in condensed milk. May it not be that the "absence" of invert sugar has been at any rate in part due to the lack of a satisfactory method for its detection? The report contains a detailed examination of a sample that gave results indicating over two per cent. of invert sugar. It may be found with improved methods of examination that the presence of invert sugar is a more common occurrence than has been thought.

This question of invert sugar is of great importance: firstly, because of its influence upon the validity of the method of arriving at the total milk solids, and secondly, because of its possible evidential value concerning the conditions of manufacture. This latter point is emphasised in a prefatory note to the Report by the Chief Medical Officer, Ministry of Health, a note which, by the way, ignores the contributions of laboratories other than that of the Ministry to the solution of the problems raised by the Condensed Milk Regulations. Sir George Newman here says "invert sugar is not a normal constituent of condensed milk, but it may occasionally be formed by the action of micro-organisms upon sucrose, and its presence is probably an indication of faulty and insanitary methods of manufacture."

There would not appear to be very much evidence, at present, to warrant this statement. Length of time of storage, referred to in the body of the Report, is equally probably an important factor. There is no evidence in the Report as to the presence or absence of invert sugar in commercial condensed milk when examined by the Barfoed process, and until further work has been done on this question it would perhaps be wise to reserve judgment.

The Report, which closes with a discussion of the interesting sample containing upwards of two per cent. of invert sugar, should, of course, be in the hands of everyone interested in the analysis of condensed milk. A detailed abstract of the methods used is given below.

Sucrose and Lactose by Polarimeter.—Dissolve about 30 grms. of condensed milk, accurately weighed, in warm water; transfer to a 250 c.c. flask, adding water to make about 100 c.c. Add very gradually, with shaking, about 50 c.c. of dialysed iron (Pharmaceutical Codex); place the flask in boiling water and gently agitate for three minutes; cool and make up to 250 c.c. with water. Mix well and filter. Allow filtrate to stand for several hours or overnight, and polarise (direct reading A).

Measure 50 c.c. of the filtrate into a 100 c.c. flask, add 10 c.c. of 5 N hydrochloric acid, immerse in water bath at 60° C. for 12 minutes, agitating the flask during the first four minutes. Cool rapidly, make up to 100 c.c. with water, allow to stand for several hours and polarise (invert reading A).

Wash the iron precipitate on the filter with a boiling 1 per cent. solution of common salt, keeping the liquid on the filter in continuous agitation by means of a jet of steam. Complete freedom from sugar (ascertained by polarimetric observation on the washings) can usually be attained in one hour or less.

Dissolve 10 grms. of sucrose and 3·5 grms. of lactose hydrate in a little warm water in the original 250 c.c. flask, which has previously been well washed out with hot water. Transfer the iron precipitate from the filter to a dish and rub up with water to a uniform cream; transfer to the 250 c.c. flask. Immerse the flask in boiling water as before, cool, make up to 250 c.c., filter, allow to stand for several hours, and proceed as before to obtain polarimetrically direct reading B and invert reading B. The actual temperature at which the polarimetric readings are taken is immaterial, provided it is the same for the two direct, and the two invert readings. If much invert sugar is present, however, the temperature should be as nearly as possible 20° C.

The change on inversion is obtained by multiplying the invert reading by two, and subtracting the product from the direct reading.

$$\text{Then sucrose per cent.} = \frac{S_2 \times P_1}{P_2} \times \frac{100}{W}$$

where S_2 is the weight of sucrose taken for second polarisation; P_1 the change on inversion of filtrate A; P_2 the change on inversion of filtrate B; and W the weight of sample taken, and

$$\text{lactose hydrate per cent.} = \frac{1619A}{WB} - 1{\cdot}269\ S$$

where A is the direct rotation of filtrate A; B the direct rotation of filtrate B; W the weight of condensed milk taken; and S the percentage of sucrose found.

LACTOSE BY COPPER REDUCTION.—Decalcify 30 c.c. of filtrate A by adding 0·06 grm. of solid potassium oxalate, shaking for 5 minutes, allowing to stand for a short time, and filtering. Dilute the filtrate to 10 times its volume with water. Dilute filtrate B ten times without decalcification.

Measure 50 c.c. of each of the diluted liquids into rubber-stoppered 100 c.c. centrifuge tubes, and add 45 c.c. of Fehling's solution (Brown Morris and Millar formula). Insert the stoppers lightly and place for 15 minutes in a boiling water bath. Remove, cool, and, after removing the stoppers, separate the cuprous oxide by centrifuging. Wash with water in the centrifuge and dissolve the cuprous oxide in 10 c.c. of a solution containing 100 grms. of iron ammonium alum and 60 c.c. of concentrated sulphuric acid in 300 c.c. of water. Directly the solution is complete titrate the liquids with $N/10$ permanganate (which for this determination need be only approximately $N/10$).

$$\text{Then lactose hydrate per cent.} = \frac{3{\cdot}5 \times V_1}{V_2} \times \frac{100}{W}$$

where V_1 is the permanganate titration of filtrate A; V_2 the permanganate titration of filtrate B; and W the weight of condensed milk taken.

If invert sugar be present, the lactose figure must be suitably corrected, for which see later under Invert Sugar.

SUCROSE BY COPPER REDUCTION.—The two filtrates, A and B, are prepared as given above for the polarimetric determination.

Decalcify 100 c.c. of filtrate A by adding 0·2 grm. of potassium oxalate and filtering after standing a short time. Invert 50 c.c. of decalcified filtrate A and of

untreated filtrate B, as before, and make up to 100 c.c. Dilute the four solutions thus obtained tenfold, and determine the copper reducing power on 50 c.c. of each diluted solution as before, the two uninverted solutions being done side by side, and the two inverted solutions similarly.

Since the reduction curve for different concentrations of invert sugar is not linear, the permanganate titrations will not stand in direct proportion to the amounts of invert sugar present. One c.c. of *N*/10 permanganate=0·00796 grm. of CuO, and the amounts of invert sugar corresponding to the amounts of CuO should be obtained by reference to Woy's tables (for 50 c.c. Fehling) (*Handbook of Sugar Analysis*, C. A. Browne).

If v_1, v_2, v_3, v_4 are the volumes in c.c. of the *N*/10 permanganate titrations of filtrate A uninverted, filtrate A inverted, filtrate B uninverted and filtrate B inverted respectively,

then $$\left(v_2 - \frac{v_1}{2}\right) \times 0{\cdot}00796 = \text{CuO} = x \text{ grms. invert sugar}$$

and $$\left(v_4 - \frac{v_3}{2}\right) \times 0{\cdot}00796 = \text{CuO} = y \text{ grms. of invert sugar.}$$

x and y being obtained from Woy's tables.

Then sucrose per cent. $= \frac{10 \times x \times 100}{y \times W}$, where W is the weight of sample taken.

INVERT SUGAR.—By comparison of the lactose figure, arrived at on the one hand by the polarimeter and on the other hand by copper reduction, it should be possible to detect 0·5 per cent. of invert sugar.

The uncertainties attaching to this method are discussed. It was ascertained that under the conditions adopted invert sugar reduces 1·3 times as strongly as lactose hydrate, but for the method of calculating invert sugar from the polarimetric readings and the copper, reference should be made to the Report. Working as described, the calculations are much simplified. Shortly, the percentage of invert sugar is the difference between the percentages of "apparent" lactose by copper reduction and by polarimeter, divided by 1·68.

The true percentage of lactose hydrate is then found by multiplying the invert sugar by 1·3 and subtracting the product from the percentage of apparent lactose found by copper reduction.

A method for the direct detection and determination of invert sugar by the application of Barfoed's solution is suggested. The method is as follows:—

Measure 25 c.c. of the condensed milk filtrate A and 25 c.c. of a similar filtrate prepared from fresh milk, with the addition of an approximately equivalent amount of sucrose, into two 100 c.c. glass centrifuge tubes, and add 70 c.c. of Barfoed's solution* to each. Place the tubes side by side in a beaker of boiling water. When the first sign of reduction is observable in the tube containing the fresh milk filtrate (usually in from 8 to 10 minutes), remove the tubes, cool quickly, separate the cuprous oxide and wash in the centrifuge, and titrate the cuprous oxide by solution in iron alum and oxidation with *N*/10 permanganate, as described under

* The solution contains approximately 50 grms. of cupric acetate and 7.5 c.c. of glacial acetic acid in 750 c.c., the copper acetate being dissolved without heating and the solution filtered clear.

the determination of lactose. The following table gives the results of trials:—

Invert sugar added (calculated as percentage on a condensed milk).	$N/10$ permanganate required.		
	Milk with sucrose and invert sugar.	Milk with sucrose alone.	Difference.
	c.c.	c.c.	c.c.
Nil	6·10	6·25	0·15
0·05	6·45	5·40	1·05
0·1	8·25	6·50	1·75
0·2	8·60	6·05	2·55
0·3	9·60	5·80	3·80
0·4	11·30	6·00	5·30
0·5	11·80	6·30	5·50
0·7	14·30	6·10	8·20
1·0	18·65	5·85	12·80
2·0	24·80	6·10	18·70

NOTE.—In all the above copper reductions, the cuprous oxide is separated by centrifuge; this procedure was found to give the most consistent results, but separation by filtration should be satisfactory.

E. HINKS.

Ceylon.

REPORT OF THE GOVERNMENT ANALYST FOR 1929.

IN his annual report the Government Analyst (Mr. C. J. Symons) states that 4046 articles were examined during the year 1929, of which 1525 were in connection with criminal investigation work, 108 for the Excise, 232 for the Customs, and 1491 were miscellaneous samples for various departments.

MILK.—The total number of samples examined was 262, as compared with 321 in 1928. Of the 49 samples from out-stations, where food supplies are under the immediate control of Local Authorities, only 13 were genuine. Added water was present in 115 of the total samples, in amounts ranging from 25 to over 80 per cent.

The absence of general legal standards and definitions necessarily hampers any attempt to remedy matters under present conditions, and there is an urgent need for a Pure Foods Ordinance for the standardisation and definition of foods and the checking of adulteration. Under the present conditions money could be saved and risks avoided by consuming imported condensed and preserved milks in preference to the local article. Milk is not the only food persistently adulterated; pepper, for example, is constantly adulterated with ground rice.

TOXICOLOGICAL CASES.—In the report for 1928 it was pointed out that the occurrence of three cases of prussic acid poisoning was probably due to the prominence given in the press to the rapidity of death and the ease with which the poison could be procured. In 1929 four times as many cases have been dealt with, indicating a remarkable development on the lines suggested. Two cases, however, were due to poisoning by manioc yams, and were probably unintentionally caused by ignorance as to the method of eliminating the cyanogenetic glucoside during cooking.

Modecca palmata Poisoning.—The seeds of this plant, which also contains a cyanogenetic glucoside, caused the death of two children who ate them. The stomach contents gave negative results in the test for cyanide, but this is not surprising in view of the results obtained in the recorded cases of such poisoning.

PROHIBITED DRUGS.—The number of samples examined was 336, as compared with 230 samples in 1928. Ganja was found in 206 samples, as compared with 77 in 1928.

Erythroxylum novogranatense.—One of the drug samples submitted was found to contain cocaine and to consist of parts of the plant, *Erythroxylum novogranatense*, presumably grown locally.

SHOOTING CASES.—There were 20 cases concerned with fire-arms, bullets, etc., and involving the examination of more than 70 articles. In several of these the question arose whether the weapon had been recently discharged.

Other shooting cases involved the examination of pieces of cloth used as wads and found at the scene of the shooting. In several cases it was found possible to show that these were not connected with pieces of cloth found in suspected persons' houses: in one case the connection of the wad with a particular piece of cloth was proved without any possibility of doubt and demonstrated photographically. In another case it was possible, by means of hairs found in the wad, to show that it must have formed part of a squirrel's nest such as was found in the accused person's house.

Two cases involved the question of the distance at which a fire-arm was discharged to cause certain marks on clothing. In one cases it was a shot gun, and in the other a revolver. In each case it was found possible by means of experimental discharges to determine the range within a very small margin.

Until this year reliance had to be placed almost entirely on a chemical examination of the contents of the barrel, together with an examination of the muzzle and working parts. Recently, however, a piece of apparatus, made by Fuess of Germany, has been introduced, whereby the inside of the barrel of a gun or other weapon can be examined directly and photographed if necessary. It consists of a long thin metal tube, with electric light bulb, prism, and mirror at one end, and an eye piece at the other; a photographic camera can be attached. By this means it is possible to make a minute examination of any part of the inside of the barrel of a gun, muzzle-loading or otherwise, or the rifling of a rifle, or the setting of the chambers of a revolver with regard to the barrel, to mention only a few uses. It is hoped that the use of this apparatus will enable accurate information to be accumulated as to the state of gun barrels at various periods after firing.

In other cases it has been of the greatest importance to determine whether or not a particular bullet, found at the scene of a crime, was discharged from a particular rifle or revolver. Until recently it has been necessary to rely upon comparison by means of photographs, showing only one aspect of the rifling. During this year, however, a piece of apparatus has been designed and made for us by Mr. J. Gray, the Government Factory Engineer, on our suggestion, whereby the two cartridges are held in line, and can be adjusted separately, but can be revolved together at the same rate. Thus, by means of a comparison microscope, consisting of two parallel microscopes with one eye piece, bringing up the two fields of view side by side, we are able to examine the marks on the two bullets, so that the riflings on the whole circumference can be compared not only at one level but throughout their length if desired.

The comparison microscope described above is also a most useful adjunct. Recently we have used it to obtain a photographic comparison between the impression of cloth on the end of a lead bullet picked up at the scene of a crime with the impression on an experimental bullet fired by us through the coat worn by the wounded man.

COUNTERFEIT NOTES AND COINAGE.—Seventeen cases of counterfeiting currency notes were investigated. Most of these were pen-drawn forgeries, but showed considerable ingenuity in producing the appearance of water marks, postal cancellations, &c. In one case the Rs. 100 note had been printed from two blocks, one for the black portion and one for the green. Crude apparatus for printing the serial numbers was seized and it was shown that the process used in preparing the blocks was a photographic one; but the only cameras found were two crude home-made affairs.

Seven cases of counterfeiting coins were investigated. A favourite method of producing a mould appears to be by compressing a genuine coin between two flat pieces of cuttle-fish bone. The resulting coin appears to pass muster after treatment.

EXAMINATION OF DOCUMENTS.—The examination of questioned documents was first undertaken by the Government Analyst in 1919, and is paid for by special fees, 10 per cent. of which, in private cases, must be credited to the revenue. The examination of handwriting was first undertaken in 1925.

Some of the cases have shown ingenuity on the part of the forgers, although in most cases the evidence from the handwriting was quite clear.

In one case, in order to be able to produce evidence that a certain amount of money had been handed over, a post card was produced with an acknowledgment of the transaction and bearing a perfectly genuine signature. In this case the fraud had been attempted by obtaining a post card written by the party on some other occasion, and then bleaching out the body of the original writing and inserting over the genuine signature what was necessary to support the case. The body of the writing certainly differed from the signature, but this was accounted for by the defence by the statement that this writer was in the habit of getting a clerk to write the bodies of his letters, merely adding the signature himself.

In another case the fraud was exposed by the discovery that a Ceylon stamp with a script CA watermark was used on a document dated before such stamps with script watermarks were issued.

Federated Malay States.

ANNUAL REPORT OF THE INSTITUTE FOR MEDICAL RESEARCH FOR 1928.

CHEMICAL examinations for the various Government Departments are made in the Chemical laboratories of the Institute, under the direction of Mr. R. W. Blair, F.I.C. The total number of samples examined was 9831, as compared with 13,558 in 1927.

MILK.—The standards prescribed for milk in the Sale of Food and Drugs Enactment, 1913, are not less than 3·25 per cent. of fat, and not less than 8·5 per cent. of solids-not-fat. The samples are taken by officers of the Health Branch.

Of the 631 samples examined, 11 contained less than the statutory amount of fat, and 84 less than the required solids-not-fat.

TODDY.—All of the 88 samples examined complied with the legal standards (see ANALYST, 1929, **54**, 290), but 22 samples, although of statutory composition, gave indications of adulteration with water.

VITAMIN *B* EXTRACT.—A method of concentration by adsorption of vitamin *B* on acid clay (see ANALYST, 1929, **54**, 562) was successfully based on the method devised by Jansen and Donath in Java.

Sifted rice polishings (1200 grms.) were extracted for six days with water (3840 c.c.), distilled arack (960 c.c.) and concentrated sulphuric acid (12 c.c.) in glass cylinders of 5 litres capacity, the whole being well stirred from time to time. At the end of the six days, the liquid was filtered, and the clear brown filtrate (4000 c.c.) shaken for three hours with acid clay (40 grms.). After filtering with suction, the acid clay was dried and found to have increased in weight by approximately ten per cent.

One grm. of this dried product, which was indistinguishable in appearance from the acid clay before treatment, was considered to be equivalent in vitamin content to 27 grms. of rice polishings.

The beri-beri preventing properties of this product were tested by the Bacteriological Division by feeding to fowls. A suspension of the treated clay in distilled water (1 grm. of acid clay in 9 c.c. of water) was made. Doses of this suspension were given daily over a period of eight weeks to nine cocks, who were fed on a diet of autoclaved Siam rice No. 1. Three cocks received a dose of 1·9 c.c. of the acid clay suspension, three received a dose of 2·2 c.c., and the remaining three a dose of 2·5 c.c. per diem.

Two of the birds in the first group (receiving 1·9 c.c.) showed a progressive loss in weight and one bird an increase in weight. None developed definite polyneuritis.

The three birds receiving 2·2 cubic centimetres per diem showed no appreciable loss in weight and remained healthy throughout the experiments.

A more complete extraction of the vitamin from the rice polishings was effected by slightly increasing the acidity of the extracting liquid. Under these conditions the efficiency by the absorption was at least 90 per cent.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Catalase Reaction of Milk. P. Weinstein. (*Z. Unters. Lebensm.*, 1930, **59**, 514–515.)—Abnormally high catalase values (*cf.* ANALYST, 1929, **54**, 237) were obtained for a sample of heated milk (63 to 65° C.) after 24 hours, though the other tests for heated milk (*loc. cit.*) were normal. This was traced to an accidental addition in the dairy of about 500 c.c. of raw milk to 400 litres of the heated milk. The catalase value of heated milk after 24 hours should not exceed 10. J. G.

Test for Amylase in Milk. P. Weinstein. (*Z. Unters. Lebensm.*, 1930, **59**, 513–514.)—The importance of the use of pure, untreated potato starch in the author's amylase test for heated milk (ANALYST, 1929, **54**, 237) has been demonstrated. A sample or Merck's soluble starch labelled "dried according to Zulkowsky," of unknown origin but obviously prepared chemically, gave negative results.

J. G.

Variations in Contents of Sugars and Related Substances in Olives. P. F. Nichols. (*J. Agric. Res.*, 1930, **41**, 89–96.)—The fruit of seventeen varieties of olives grown in California was examined with regard to the amount and nature of the reducing substances in the pulp and the relation of these to the maturity and variety of the olives. Clarification of the solution by basic lead acetate was found unsatisfactory when sugars were to be determined by the picric acid method. Mercuric nitrate was used for the purpose, and the reducing substances in the aqueous extract, before and after hydrolysis, were removed by a short treatment with yeast, and appeared to consist entirely of fermentable sugars. The mean early season ratio of simple and total sugars (expressed as dextrose) was 0·86, and for the late season 0·77. The amounts of sugars (entirely or mostly sucrose) hydrolysed by heating with picric acid, usually constituted 15 to 25 per cent. of the total reducing sugars. The trend is for the sugar content to increase in October and November, and decrease in December and January. Roughly, the varieties examined constituted three groups: (1) High sugar content: Ascolano, P.I.G. 27172, Barouni and Sevillano; (2) medium sugar content: Mission, Chitoni, Manzanillo, Chemali, Columbella, Nevadillo, Bidh el Hammam, Saiali, Magloub, and Uvaria; (3) low sugar content: S.P.I. 27173, Lucca, Picholine, and Salome. D. G. H.

Detection of Carrots in Marmalade. A. Hanak. (*Z. Unters. Lebensm.*, 1930, **59**, 513.)—As a rapid sorting method it is suggested that the marmalade (or jam) should be thoroughly broken up and extracted with ether, and the extract allowed to evaporate on a clock-glass. In the presence of carrots characteristic yellow rings of carotene are obtained, which are readily distinguishable from the coloured constituents natural to jam. J. G.

Acidity and Corrosion in Canned Fruits. E. F. Kohman and N. H. Sanborn. (*Ind. Eng. Chem.*, 1930, **22**, 615–617.)—Although the effect of *p*H value on the tin or the base plate may be definite, it is not always possible to predict the exact effect, but there is a general tendency for tin to become relatively less oxidisable than iron with decrease in *p*H value within the range encountered in fruits, and probably beyond. In a plain can the tin is the anodic metal, and its area compared with that of the iron is huge, which explains its protective effect, but in an enamelled can both iron and tin are exposed to the contents, due at first to rupture of the enamel seam, and the relative area of the cathodic iron is enough to have an appreciable effect on the corrosion of the tin when it is exposed, and local couples form. If the tin is rendered still less oxidisable by the addition of citric acid, perforation of a tin can may be inhibited. One per cent. citric acid

entirely inhibited perforation in an experiment lasting 302 days. Dehydrated prunes were found to be far more corrosive than fresh owing to the lye dipping which somewhat affects the *p*H value, reducing the acidity. The fruits which cause the greatest amount of perforation are those with the lowest acidity or highest *p*H value. The effect of different acids is not exactly comparable in rendering tin anodic to iron, but citric acid has a more pronounced effect than malic acid. A table shows the effect of varying additions of acids in equivalent amounts and of alkali in the case of dehydrated prunes. D. G. H.

Determination of Copper in Green Vegetables. A. Hanak. (*Z. Unters. Lebensm.*, 1930, **59**, 511–512.)—It is suggested that for samples which are not easily ashed by direct heat, 10 grms. should be warmed with 25 drops of concentrated sulphuric acid, heated until dry, and the residue charred, broken up and extracted twice with warm (1: 5) nitric acid. (Platinum dishes are not recommended for this purpose.) The residue is then completely ashed, dissolved in the acid extract and an excess of ammonia added followed by ammonium carbonate, and the whole warmed and filtered. The residue from the filtration is washed with a hot dilute solution of ammonia and ammonium carbonate, redissolved in a little dilute acid, reprecipitated and filtered. The filtrates are combined, and the blue colour matched directly with ammonia and standard copper sulphate solution, or if the amount of copper is very minute the ferrocyanide method is used in the presence of a solution of gum arabic. The maximum error in experiments in which 0·5 to 0·9 mgrm. of copper were added to samples of peas, beans and spinach was 0·02 mgrm. J. G.

Microscopical Pollen Analysis of Honey. C. Griebel. (*Z. Unters. Lebensm.*, 1930, **59**, 441–471.)—The third of a series of descriptions, illustrated by photo-micrographs, of the microscopical characteristics of the pollen grains of 181 plants, which may be found in honey of German origin. Previous communications (*id.*, 1930, **59**, 63, 77, 197) deal with the Dicotyledons, and the present paper with the Monocotyledons, which are distinguished by one instead of three folds, forming a deep furrow along one side of the usually elliptical grain. J. G.

Colorimetric Method for the Determination of the Amount of Potassium Ferrocyanide required for Fining Wine. A. Hanak. (*Z. Unters. Lebensm.*, 1930, **59**, 506–511.)—Twenty-five c.c. of the wine are placed in each of the two containers, A and B, of a colorimeter (B being the standard for comparison), equal quantities of an aqueous solution of iron-free gum arabic added to each to prevent flocculation, and 3 to 5 drops of 0·05 per cent. ferric chloride solution in dilute hydrochloric acid then added to B. A measured excess of a fresh 0·5 per cent. solution of potassium ferrocyanide is then added to A (usually about 0·6 c.c.) so as to cause all the iron to react. The same amount is then added to B, and 2 drops of 30 per cent. hydrogen peroxide or dilute nitric acid and 5 c.c. of hydrochloric acid (1:5) added to each container, the latter being required only if there is a tendency for the iron to become insoluble. The contents of B are now diluted

with iron-free wine or water till an approximate colour-match is obtained, a yellow dye added to compensate for any slight differences in shade, and a final match obtained. If x is the volume of ferrocyanide solution used, and a and b the volumes of liquid in A and B, respectively, then the volume of ferrocyanide solution required for fining is ax/b c.c. With red wines it is preferable to determine the iron in the ash colorimetrically and to obtain the equivalent of ferrocyanide reagent by calculation. The method may also be used for the determination of iron in wine.

J. G.

Determination of Essential Oil of Mustard in Black Mustard. R. Meesemaecker and J. Boivin. (*J. Pharm. Chim.*, 1930, **122**, 478–484.)—Five grms. of finely powdered mustard, passing a No. 9 sieve, and 100 c.c. of water are macerated for 1 hour at 68° C., after which 100 c.c. of ammonia (French officinal, *i.e.* 20·18 per cent. of NH_3) are added and left for 15 minutes. On cooling, the mixture is made up to 200 c.c., and 20 c.c. of *Courtonne's* reagent (a neutralised 30 per cent. solution of lead acetate) added, and 100 c.c. of filtrate neutralised and then acidified with 5 c.c. of 0·5 N sulphuric acid and 10 c.c. of 0·1 N iodine solution added. After 15 minutes' contact in a stoppered flask about 10 c.c. of benzene are added, and the excess of iodine titrated with 0·05 N thiosulphate solution. One litre of 0·1 N iodine solution is equivalent to 5·8 grms. of thiosinamine or 4·85 of essential oil of mustard. This method gave results agreeing very closely with those by the supplementary French Codex method of 1925, and it was found that the proportion of essential oil of mustard diminishes with increase of time of maceration. With mustard flours from which the oil has been extracted the maximum yield is obtained at 40° C. D. G. H.

Detection and Determination of *p*-Hydroxybenzoic Acid and of its Esters in Foodstuffs. F. Weiss. (*Z. Unters. Lebensm.*, 1930, **59**, 472–480.)—The method of extraction previously described (ANALYST, 1928, **53**, 291) gives 100 per cent. yields for non-fatty samples, and 70 to 90 per cent. for fatty foods. The methyl ester is detected in the manner described (*loc. cit.*), but for the ethyl or propyl ester the separated extract is heated under a reflux condenser for 1 hour with 4 c.c. of water and 2 c.c. of 10 per cent. sodium hydroxide solution, and 4 c.c. distilled into a receiver cooled in ice with precautions appropriate to the prevention of loss of alcohol. Two c.c. of distillate are used for the test for methyl alcohol (*loc. cit.*), and 2 c.c. are oxidised with 5 drops of 50 per cent. chromic acid solution, and the resulting aldehyde identified from the *m*- and *p*-nitrophenylhydrazones (Griebel and Weiss, *Z. Unters. Lebensm.*, 1928, **55**, 31; **56**, 160; and *Mikrochemie*, 1927, **5**, 146). The acid is detected by heating the residue from the original ethereal extraction for 1½ hours with 7 per cent. sodium hydroxide solution under a reflux condenser, extracting the solution with ether after acidification with dilute sulphuric acid, rendering the ethereal extract alkaline, and distilling off the ether. The residue is then acidified, re-extracted with ether, and the final extract dried over sulphuric acid. The acid has m.pt. about 212° C. (recorded 215° C.), and gives a red colour with Millon's reagent. When dissolved in ammonia, the excess of

which is removed by warming, and precipitated with copper sulphate solution, it forms a copper salt in bright blue needles, sparingly soluble in water or alcohol. The separation of the ester is unaffected by the presence of benzoic, salicylic, and *o*- or *p*-chlorbenzoic acids, but vanillin should first be separated by warming the ethereal extract with water in the presence of semioxamazide, from which the ester may then be extracted in ether. The copper salt of *p*-hydroxybenzoic acid may readily be identified microscopically in admixture with those of the other acids, or, alternatively, the greater proportion of the latter may be separated by steam-distillation, or by repeated extractions on the water-bath with 20 c.c. portions of carbon tetrachloride. For quantitative purposes the purified ethereal extract may be weighed, the Zeisel method used, or the methyl alcohol determined (*loc. cit.*). If only the acid and ester are present as preservatives the extract is saponified, the solution acidified, shaked with ether, and the extract weighed, when the factors **1·1**, **1·2** and **1·3** give the amounts of methyl, ethyl, and propyl esters, respectively (1·2 for mixtures). The method was applied to sugar solutions, raspberry juice, and mayonnaise containing the above preservatives, and 62 to 99 per cent. of the *p*-hydroxybenzoic acid or ester added was recovered, while margarine yielded 63 per cent. of the preservative. "Nipagin" was found to contain 28·3 per cent. of free ester, 45·8 per cent. of its sodium compound, and 6·8 per cent. of sodium *p*-hydroxybenzoate.

J. G.

Biochemical.

Presence and Distribution of Titanium in Animals. G. Bertrand and Voronca-spirit. (*Bull. Soc. Chim.*, 1930, **47–48**, 643–646.)—Various organs of some twenty different types of animal were examined for titanium by the method used for plant tissues (*Bull. Soc. Chim.*, 1929, **45**, 1044), whereby, operating in all cases on 100 grms. of fresh material, as little as 0·3 mgrm. per kilo. is determinable. In the horse, calf, sheep and pig the liver was the organ richest in titanium, containing 0·5 to 0·6 mgrm. per kilo. of fresh material, whilst in no case was any found in the muscles. No titanium was found in any organs of the rabbit, but it was present in the hair which had been well washed 2·2 mgrm. per kilo.). It was also absent from chicken liver, but present in quantities from 0·3 to 0·9 mgrm. per kilo. of fresh material in various fish, increasing still more in crustaceans and molluscs. Mussels, taken out of the shell, showed 6 mgrms. per kilo. of fresh material and snails 0·6 mgrm. Titanium is regarded as one of the elements normally present in living matter.

D. G. H.

Traces of Combustible Gases in Human Expired Air. T. R. Parsons. (*Biochem. J.*, 1930, **24**, 585–588.)—Campbell (*J. Physiol.*, 1929, **67**, Proc. 6) showed that the gases present in the peritoneal cavities of animals may contain as much hydrogen (produced by intestinal putrefaction) as corresponds to a tension of the order of 2 mm. of mercury. Some of this hydrogen must dissolve in the blood and later diffuse through the lungs into the expired air. Attempts have now been

made to determine the extent to which such a transfer of hydrogen or other combustible gas takes place in the human subject, in order to decide whether this constitutes a factor necessary to be taken into consideration in the calculation of the total respiratory exchange from observations carried out with an open circuit respiration chamber. For the investigation the gas analysis apparatus accurate to 0·001 per cent. described by Krogh (*Biochem. J.*, 1920, **14**, 267; ANALYST, 1920, **45**, 395) was available, and the technique adopted is described. The slight shrinkage in volume when pure atmospheric air is submitted to combustion analysis observed by Krogh (*Kgl. Danske Vidensk. Selsk. Math.-fys. Medd.*, 1919, **1**, 12) is confirmed; for each minute of exposure to the hot wire shrinkage occurs to the extent of about 0·001 per cent. of the original volume, and the volume of the potash-absorbable product apparently formed is always less than twice the contraction on combustion. This effect cannot be due to the presence of traces of ordinary combustible gases as it is obtained in air that has been passed over red-hot copper oxide. In order to have the maximum amount of hydrogen for measurement, the expired air as directly collected was not analysed, but air that had been re-breathed until its hydrogen tension had come into equilibrium with that in the blood. Re-breathing was stopped when the added oxygen (that added to the air in a bag to cover the needs of the subject during the experiment) had been used up by the subject; this point was easily observed. It was found that air that has been re-breathed by a human subject contains traces of combustible gases in amounts that indicate a rate of production of about 0·02 c.c. per minute. It is concluded with certainty that whatever may be the nature of these combustible expired gases the amount is far too small to be of significance in determinations of total respiratory exchange. P. H. P.

Studies on Crystalline Insulin. IX. The Adsorption of Insulin on Charcoal. H. Jensen and A. De Lawder. (*J. Biol. Chem.*, 1930, **87**, 701–712.)—Dingemanse (*Arch. Exp. Path. u. Pharmakol.*, 1928, **127**, 44; *Arch. néerl. physiol.*, 1927, **12**, 259) claimed to have obtained an insulin preparation with an activity of about 150 international units per mgrm. Du Vigneaud, Geiling and Eddy (*J. Pharmacol. Exp. Therap.*, 1928, **33**, 497) studied the adsorption of crystalline insulin on charcoal (with the use of norit as the adsorbent), and found that the product obtained after adsorption was not more active than the starting material, and that it also could again be obtained in crystalline form. They did not know, however, that Dingemanse had only obtained positive results with the use of medicinal supranorit, obtained from the Noritmaatschappij, Ltd. It was thought desirable to repeat the work of Dingemanse. Various insulin preparations—pyridine precipitate, crystalline insulin, and pig insulin—were taken, and submitted to the adsorption on charcoal according to Dingemanse, with the use of the same grade as employed by Dingemanse, and no product more active than crystalline insulin was obtained; similarly no preparation more active than crystalline insulin was obtained when the work of Dingemanse was repeated with the use of the same insulin preparation and the same charcoal as employed by Dingemanse. In agreement with Dinge-

manse it was found that the final preparation obtained from organon is rather unstable, and loses most of its activity whether kept in solution or in dry form. The results obtained do not substantiate the claim of Dingemanse to be able to obtain an insulin preparation which is more active than crystalline insulin. Dingemanse, in a recent personal communication to the authors, states that one has to use a certain amount of supranorit for a given quantity of insulin, and with different insulin preparations one always has to find out first the exact amount of supranorit to be used in order to obtain positive results. The authors have published their results in the hope that other laboratories will be induced to repeat the work. They consider that the experimental conditions which should give positive results must be put on a much sounder basis before the results of Dingemanse can be generally accepted. P. H. P.

Biological Study of Sterols. Sterols of Plankton. G. Belloc, R. Fabre and H. Simonnet. (*Compt. rend.*, 1930, **191**, 160–162.)—Plankton, taken in the Bay of Bourgneuf in hot calm summer weather, consisted of larvae of *Porcellana*, Copepods of the genus *Calamus*, and some rare Ctenophores of the genus *Cydippes*, whilst sample No. 2, taken in rough cold spring weather, consisted exclusively of Cydippes with rare Beroes. The samples were filtered through cotton wool, the plankton put into a large excess of acetone, and samples preserved in dilute (1:1) formalin. The sterols were isolated by filtering the acetone solution and extracting the residue with acetone and chloroform. After distilling off the solvents *in vacuo* the residue was saponified, and the unsaponifiable matter crystallised from methyl alcohol. These operations were conducted in the absence of air, and, as far as possible, of light. The product was examined by ultra-violet absorption, the colour reaction of Rosenheim and Meesemaeker, and biologically. Sample No. 1 (total weight 0·154 grm.) showed the characteristic absorption bands of ergosterol and its chemical reactions, and a dose of 0·01 mgrm. of the sample, without irradiation, gave a positive result on rachitic rats. Sample No. 2 (total weight 0·145 grm. with uncrystallisable residue 0·687 grm.) gave negative results in tests for ergosterol and even for a rachitic rat before irradiation, with a positive test after 45 minutes' irradiation in a dose between 0·01 and 0·1 mgrm. Sterols extracted from medusae of *Acalephes* were similar in behaviour to sample No. 2. The biological activity of planktons is regarded as a function of many factors, but principally of light and the zoological nature of the material. D. G. H.

Preparation and Antirachitic Activation of some Derivatives of Ergosterol and Cholesterol. D. W. MacCorquodale, H. Steenbock and H. Adkins. (*J. Amer. Chem. Soc.*, 1930, **52**, 2512–2518.)—A number of ergosterol and cholesterol derivatives, the preparation of which is described, have been subjected to the radiation of a Hanovia Alpine Sun Lamp at a distance of 25 cm. to ascertain if antirachitic activity was developed in this way. Ergosterol-*d*-glucoside, tetra-acetylergosterol-*d*-glucoside, ergosteryl hippurate, and possibly α-ergostenol and α-ergostenyl acetate, may be activated, but negative results were obtained with cholesterol-*d*-glucoside, cholesteryl hippurate, *iso*-ergosterol and *iso*-ergosteryl acetate. T. H. P.

Mechanism of the Liebermann and Burchard Reaction. Application to the Differentiation of Animal from Vegetable Sterols. R. Meesemaecker and H. Griffon. (*J. Pharm. Chim.*, 1930, [viii], **11**, 572–580.)—In this reaction for sterols, which consists in adding acetic anhydride and sulphuric acid to a chloroform solution of the sterol, the chloroform is found to be unnecessary. Organic solvents of most varied chemical functions may be employed; alcohols alone seem to be inadmissible, since they give no reaction either when sulphuric acid or when other dehydrating agents are used. With phytosterol and cholesterol, the sulphuric acid plays a part in the colour reaction other than a mere dehydrating action, although with potassium hydrogen sulphate instead of the acid, phytosterol gives a pale green coloration which distinguishes it sharply from animal sterol. In the various solvents tried, ergosterol shows a number of colour reactions with most dehydrating agents, a green or pink coloration being mostly obtained.
T. H. P.

Method of Purifying Carotene and the Vitaminic Activity of the Purified Product. M. Javillier and L. Emerique. (*Compt. rend.*, **1930, 191,** 226–229.)—The carotene was purified by dissolving it in carbon disulphide and pouring the solution, drop by drop, into a large excess of boiling methyl alcohol, eliminating the carbon disulphide, and filtering the boiling alcoholic liquid in which the precipitated carotene is held in suspension. These operations are carried out in an atmosphere of nitrogen, and the purification may be repeated as desired. Five purifications carried out on 650 mgrms. of crystallised carrot carotene, melting at 172–173° C., and known to be active as a daily dose of 0·03 mgrm. to rats with rickets, resulted in 178 mgrms. of a body melting at 184–185° C. and still active with regard to vitamin *A* in a dose of 0·06 mgrm. per day. A dose of 0·045 mgrm. produced very similar results, and 0·03 mgrm., whilst arresting the drop in weight and curing the xerophthalmia did not uniformly produce a gain in weight.
D. G. H.

Vitamins in Oils. F. S. Gerona. (*Ann. Falsificat.*, 1930, **257,** 284–290.)—By treating oils with a mixed alcohol-ether solvent (ethersol), it is possible to detect vitamins *A*, *B* and *D* spectroscopically in the solution obtained, which shows absorption bands in the red, blue, yellow and violet. By using tubes of different widths the concentration of the vitamins may be judged. The percentages of vitamin found in brown and pale cod-liver oils and in olive oil were, respectively, 23·5, 9·3 and 12·16. Refined grape-seed oils show no trace of absorption and are hence free from vitamins *A*, *B* and *D*. Olive oil contains vitamin *B*, which does not occur in brown cod-liver oil. The refining of olive or grape-seed oil by processes which involve subjecting the oil to a temperature of 150° C. destroys the vitamins to some extent, while complete destruction takes place at 200° C. As they are not toxic, natural grape-seed oils have a greater alimentary value than brown cod-liver oil.
T. H. P.

Vitamin *A* and Carotene. V. Absence of the Liver Oil Vitamin *A* from Carotene. VI. Conversion of Carotene into Vitamin *A* in Vivo. T. Moore.

(*Biochem. J.*, 1930, **24**, 692–702.)—Some of the previous work on carotene and its vitamin *A* activity is briefly discussed. Experiments which are described show that the antimony trichloride colour reaction given by a minimal physiological dose of carotene at 590$\mu\mu$ is slightly less than that given by a minimal physiological dose of the vitamin *A* of liver oil at 610–630$\mu\mu$. It is therefore impossible that the colour reaction of carotene could conceal any underlying colour due to the liver oil vitamin *A* in such amounts as would account for its physiological activity. When, in addition, the work of Capper on the absence of the 328$\mu\mu$ absorption band from carotene is considered, the reasonable conclusion is that the activity of the pigment cannot be attributed to direct contamination with vitamin *A*, or at least with the chromogen associated with that factor in liver oils. More extensive evidence is given in support of the suggestion that a chemical relationship between carotene and the vitamin may exist. In a study of the conversion of one to the other, recourse to colorimetric data was unavoidable, since carotene and the liver oil vitamin *A* both give positive results when tested biologically. It was found that the liver oils of rats suffering from vitamin *A* deficiency invariably gave negative results when tested with the antimony trichloride reagent. Rats after depletion of vitamin *A* were cured by the administration of large excess of carotene (12 times recrystallised from cyclohexane, M.P. 178° C. uncor.), red palm oil, or fresh carrots. Traces of yellow pigment then appeared in the liver oil, but the predominant chromogen present (99 per cent.) was invariably vitamin *A*, as characterised by (*a*) absence of such intense yellow pigmentation as must have accompanied the storage of carotene as such; (*b*) an intensely positive antimony trichloride reaction showing a marked band at 610–630$\mu\mu$; (c) the development of an absorption band in the untreated oil at 328$\mu\mu$; (*d*) intense biological activity. The conclusion must therefore be reached that carotene, or some part thereof, if it should later prove to be heterogeneous, behaves *in vivo* as a precursor of the vitamin.

P. H. P.

Vitamin *A* Content of Ghee. A. L. Bacharach. (*Brit. Med. J.*, 1930, July 26, 141–142.)—The heating or "rendering" of ghee is sometimes followed by a "re-rendering." Ghose (*Biochem. J.*, 1922, **16**, 35; ANALYST, 1922, **47**, 267) stated that he had found vitamin *A* present in ghee, but his results were obtained before vitamin *A* had been distinguished from vitamin *D*. The author recently came into possession of some samples of Indian ghee. They arrived either in ground-glass stoppered jars or in sealed tins, and thus there is no reason to assume that their vitamin content had diminished appreciably during transit. The unsaponifiable matter from four samples was dissolved in chloroform and submitted to a modified Carr and Price test with antimony trichloride. Two showed no trace of blue colour, and only one showed a measurable quantity. This last sample was fed daily to a rat whose reserves of vitamin *A* had been depleted on a diet adequate in other respects. It was found that 5 mgrms. of cod-liver oil was markedly superior to 200 times the quantity of ghee (the sample shown by the colour reaction to be superior to the other three samples). Therefore for

practical purposes this ghee must have been devoid of vitamin *A*; the other three samples must have been at least as deficient. The samples were selected as typical of those to be obtained in the open market, and it is suggested that the problem needs some fuller and more official investigation. P. H. P.

The Fat-Soluble Vitamins of Milk. M. E. F. Crawford, J. Golding, E. O. V. Perry and S. S. Zilva. (*Biochem. J.*, 1930, **24**, 682–691.)—McCollum, Simmonds and Steenbock (*J. Biol. Chem.*, 1917, **29**, Proc. 26) observed that fat-free milk, when included in a diet consisting of purified food substances, promoted growth and prevented decline of animals, and, further, that melted butter-fat lost its growth-promoting capacity after being thoroughly agitated with twenty successive portions of water. This led them to suspect that the "fat-soluble *A* factor" might be appreciably soluble in water, although Steenbock, Boutwell and Kent (*J. Biol. Chem.*, 1918, **35**, 517) later demonstrated that it was destroyed in the process of washing. No strictly quantitative experiments have since been carried out to find whether the growth with the "fat-free" milk observed by McCollum, Simmonds and Steenbock was due to the presence of the fat-soluble vitamins present in the residual fat of the skim milk, which it is presumed their milk contained, or whether one or both of the fat-soluble vitamins of milk-fat is really appreciably soluble in water, and an enquiry has now been made to establish this point. Rats were used for the tests. The results suggest definitely that the entire vitamin *A* and vitamin *D* content of milk is associated with the milk fat. There are no indications that these vitamins are more concentrated in one fraction of the milk-fat than in another, nor that there is a significant loss in either vitamin due to separating or churning, and consequently by testing the butters it is possible to obtain the approximate relative potency of milks. The experiments further suggest the utility of the Soames and Leigh-Clare method (*Biochem. J.*, 1928, **22**, 522) for the determination of vitamin *D*. The limitations of this method are being studied further. P. H. P.

The Jansen and Donath Procedure for the Isolation of Antineuritic Vitamin. R. R. Williams, R. E. Waterman and S. Gurin. (*J. Biol. Chem.*, 1930, **87**, 559–579.)—Jansen and Donath (*Mededeel. Dienst Volksgezondheid Nederland.-Indië*, 1926, pt. 1, 186) described a somewhat complicated procedure, based largely on the use of reagents previously suggested by Funk and Seidell, by means of which a pure crystalline substance of antineuritic properties, and of the composition $C_6H_{10}N_2O.HCl$, had been obtained from rice polish; this final product was also tested on a few pigeons. The result was complete prophylaxis against polyneuritis, and a high degree of maintenance of appetite, but accompanied by moderate losses in weight unless the diet was supplemented with extracted meat powder. The discovery by Williams and Waterman (*J. Biol. Chem.*, 1928, **78**, 311; ANALYST, 1928, **53**, 505) of the existence of a "third factor" necessary for weight maintenance in pigeons gave a more favourable significance to the weight losses, on account of which there had been some doubt as to whether the isolated crystals were the long-sought antineuritic vitamin or not. It was therefore decided to repeat the

chemical work of Jansen and Donath in detail and to carry out extended feeding tests upon both rats and pigeons not only with the final product but also with each of the principal fractions discarded. It was found that the process of Jansen and Donath (with minor modifications) applied to brewers' yeast leads to heavy losses of activity and a negligible recovery of active material in the selected fraction after platinum chloride precipitation. The same process (with slight variations) applied to rice polish leads to smaller losses and better recovery, but only one-fifth as much activity was present at the platinum chloride stage, as was reported by Jansen and Donath. Further fractionation with alcohol and acetone did not yield crystals as described by Jansen and Donath, but the relative distribution of activity in various fractions corresponded roughly with their report. Fractionation of a by-product with reagents mentioned by Jansen and Donath betrayed no error in their account of the precipitation reactions of the vitamin, and yielded a non-crystalline material of substantially the same physiological properties as the Jansen and Donath vitamin. Vitamin hydrochloride crystals supplied by Jansen and Donath were tested on pigeons on a polished rice diet; in doses of 0·04 mgrm. daily, polyneuritis and death were much retarded, but weight loss was not avoided. Rats on a synthetic diet including autoclaved yeast showed a marked growth response to daily doses of 0·04 mgrm. of the same crystals. The more advanced fractions of the authors also react qualitatively and approximately quantitatively in the same way as the crystals of Jansen and Donath upon both rats and pigeons. The authors conclude that the crystals of Jansen and Donath (whether pure or not they cannot say) possess antineuritic properties; they are inclined to take the Dutch workers' claims of isolation seriously. The antineuritic vitamin in small doses does not affect the weight curves of pigeons on polished rice. Birds on this diet suffer from a multiple deficiency. To what extent reliance on weight curves has affected earlier conclusions can be estimated only very roughly, but some experiments must have been adjudged failures because they were chemically successful, *i.e.* actually eliminated some impurity of favourable physiological effect but foreign to the antineuritic vitamin itself. Peters and his associates have preferred for years past to measure activity by length of delay of recurrence of symptoms after administration of a curative dose, and have not relied considerably on the weight curves. P. H. P.

The Williams-Waterman Vitamin B_3. W. H. Eddy, S. Gurin and J. Keresztesy. (*J. Biol. Chem.*, **1930**, 87, 729–740.)—New evidence is submitted to demonstrate further the existence of a bird growth and weight restoration factor provisionally designated by Williams and Waterman as vitamin B_3, and distinct in distribution and properties from the antineuritic factor (vitamin B_1 or B) or the antipellagric factor (vitamin B_2, G, or P-P). Pigeons were placed on polished rice until well advanced toward polyneuritis, then an amount of vitamin B_1 adequate to prevent the polyneuritis was added to the diet. After 10 to 14 days on the B_1 supplement alone, measured amounts of the food to be assayed were added, and their effect on weight restoration noted. The extent of weight increase indicated

relative richness of the foodstuff in vitamin B_3 or whatever is the weight-restoring factor. In some cases the B_1 supplement was omitted at a later stage to determine whether the foodstuff possessed adequate B_1 itself. The results, which are tabulated, indicate among other things that certain fairly good sources of B_1 are not equally rich in B_3 and *vice versa*. Yeast, whole grains and malt are good sources of vitamin B_3, but whilst malt extract often retains a good concentration of B_1, its manufacture practically eliminates B_3. Beef and beef liver are fair sources of B_3, and distinctly superior in this factor to milk, orange and tomato juice, spinach, and potato juice or cane molasses. Vitamin B_3 is a bird requirement, for in none of the tests was it found possible to show the need of vitamin B_3 on the part of rats. Chicks require this factor for growth, even when fully supplied with B_1 and B_2 factors and with other nutrients and vitamins. It was found that baking corn and autoclaving chick feed destroys vitamin B_3. Vitamin B_3 is much more heat-labile than B_1, and if submitted to alkali treatment before drying, temperatures as low as 20° C. will markedly reduce the yeast content of this factor. Malt extracts made at temperatures as low as 60° C. are practically devoid of vitamin B_3 though still very effective as sources of vitamin B_1. P. H. P.

Nature of Vitamin *C*. Study of its Electrical Transference. R. B. McKinnis and C. G. King. (*J. Biol. Chem.*, **1930**, **87**, 615–623.)—The instability of vitamin *C* results in loss of the antiscorbutic value of foods and makes the study of the vitamin highly desirable, but the same property makes experimental work very difficult. Destruction is due chiefly to oxidation and is favoured by heat, catalysts and alkalinity. If, in an acid solution, an electric current transfers the vitamin toward the cathode, the vitamin is either a true base or an ampholyte, but if transference takes place toward the anode it can only be a true acid. If the solution is basic and migration is towards the cathode then the vitamin is a true base, but if toward the anode, it can either be a true acid or an ampholyte. During a simple electrolysis, the conditions at either electrode would be ideal for inactivation. A new type of electrical transference apparatus, which protects the solution from alkali, oxygen, chlorine and heat liberated during electrolysis, as well as oxygen from the air, has now been devised, and used for a study of the acid-basic properties of vitamin *C*. The activity of lemon juice in the anode, cathode and middle compartments after 5 hours', electrolysis was measured for antiscorbutic value by feeding to guinea pigs. Evidence was found for the rapid diffusibility of vitamin *C* through collodion membranes. Comparative measurements were made for the transfer of amino acids in acid and faintly alkaline solutions, showing a marked concentration in the cathode chamber in acid solution and in the anode chamber in alkaline solution (*p*H 7·2). Vitamin *C* showed no distinct transference towards the cathode in strongly acid solution (*p*H 0·9), nor in natural lemon juice (*p*H 2·4). It is concluded that the antiscorbutic vitamin is not a salt-forming nitrogen compound such as an amino acid. In slightly alkaline solution there was evidence of a concentrating effect in the anode chamber, indicating that the active substance is acidic in nature. These findings are in harmony with previous evidence concerning the chemical nature of vitamin *C*. P. H. P.

Formation of Vitamin *D* by Monochromatic Light. A. L. Marshall and A. Knudson. (*J. Amer. Chem. Soc.*, 1930, **52**, 2304–2314.)—The effect of intense light from a magnesium spark or a quartz cadmium arc on an absolute alcoholic solution of ergosterol (20 mgrms. per 100 c.c.) has been investigated. The rate of production of vitamin *D* from the ergosterol is proportional to the first power of the light intensity, and is directly proportional to the number of light quanta absorbed by the ergosterol and independent of the wave-length of the light used. Vitamin *D* absorbs in the same wave-length region as does ergosterol and is destroyed by light of the same wave-length as that which forms it. The maximum possible concentration of vitamin *D* obtainable by direct irradiation of ergosterol is 35 per cent., the actual concentration attained being probably lower. The quantum efficiency of the reaction is 0·3 molecules of vitamin *D* per quantum of light absorbed. T. H. P.

Beef Extract as a Source of Vitamin G. R. Hoagland and G. G. Snider. (*J. Agric. Res.*, 1930, **40**, 977–990.)—Vitamin *G* is the more heat-stable water-soluble dietary factor of vitamin *B*, and the relative amounts present in beef extracts from five factories were determined on albino rats. Maize extract was used as the source of vitamin *B* (antineuritic). An intake level of 7·5 per cent. moisture-free beef extract from four factories furnished sufficient vitamin *G* to promote good growth, but that from the fifth factory only induced fair growth. The average intake of moisture-free beef extract was 0·8 grm. for male and 0·65 for female rats. If dried lean beef was used, the quantity required for normal growth was three to four-tenths as much. One part of beef extract appeared to contain approximately as much vitamin *G* as eleven parts of fresh lean beef.

D. G. H.

Bacteriological.

Occurrence of Typhoid-Paratyphoid Bacilli in Sewage. R. S. Begbie and H. J. Gibson. (*Brit. Med. J.*, 1930, **55–56.**)—A series of 58 samples of sewage, each from a different main sewer of the Edinburgh system, has been examined for organisms of the enteric group by the following three methods. (1) Direct plating on MacConkey's medium containing both lactose and sucrose. (2) Each of ten tubes of Brilliant green (concentration ranging from 1:100,000 to 1:2,000,000) peptone water medium (*p*H 6·4) was inoculated with a large loopful of sewage and incubated at 37° C. for 48 hours, after which from one to three loopfuls (according to the growth visible) from each tube were spread on a plate of the lactose-sucrose medium. (3) Three plates of the glucose-iron-bismuth-sulphite Brilliant green medium described by Wilson and Blair (*J. Hyg.*, 1927, **26**, 374) were inoculated with three to five loopfuls of the sewage, distributed with a glass spreader. After incubation for 48 hours, suspicious colonies were sub-cultured on to MacConkey plates, pale colonies from three sets of these being sub-cultured into tubes of peptone water and incubated for 48 hours. Tubes showing pigment production indicative of *B. pyocyaneus* or allied types were then

rejected, as were any of the remainder reacting positively to the indole test. The remaining growths were sub-cultured on a coagulated serum medium, so that after incubation for 48 hours proteolytic organisms could be excluded. Biochemical tests were then made for fermentation of glucose, lactose, and mannitol, and for formation of hydrogen sulphide in lead acetate and glucose agar. In this way it was possible to select possible strains of the enteric group from each sample with the use of the minimum of media. Identification was completed by biochemical reactions with a wide range of carbohydrates and the use of a high titre immune agglutinating serum.

Of the 58 samples of sewage, 7 yielded organisms identified as *B. paratyphosus B. Schottmüller* by direct agglutination and agglutin-absorption tests. From 5 of these samples the organism was isolated only by the Brilliant green enrichment method, and from 2 by Wilson and Blair's method; in no case was a positive result obtained by both methods. Direct plating of the sewage on MacConkey's medium gave negative results throughout. With the Brilliant green enrichment method, the most effective concentrations were from 1:200,000 to 1:400,000.

The presence of organisms of the enteric group in sewage indicates missed cases, convalescents, and transient contact carriers in the general population, and may occur in association with, or immediately following, an outbreak of the disease. It is, however, suggested that a possible source of infection may exist without any distinct outbreak of the disease either at the time of the examination or preceding it. T. H. P.

Variation of Phenol Coefficients of Coal-Tar Disinfectants with Different Test Organisms. B. G. Philbrick. (*Ind. Eng. Chem.*, 1930, **22**, 618–619.)—Four grades of coal tar disinfectants previously standardised by the Rideal-Walker method and having coefficients of approximately (*a*) 2 to 3, (*b*) 5 to 6, (*c*) 9 to 10, and (*c*) 20, were tested for germicidal action against *B. typhosus*, *Staphylococcus aureus*, *B. diphtheriae*, *Streptococcus haemolyticus*, and *Pneumococcus*. If the *B. typhosus* phenol coefficient of the disinfectant is known it is possible to calculate from the tables its efficiency against the other organisms in the presence or absence of organic matter. The phenol coefficients as determined were:—*B. typhosus*, with and without organic matter; (*a*) 3·2, 2·9, (*b*) 6·5, 5·8, (*c*) 10·0, 9·0; (*d*) 19·0, 17·0; *Staphylococcus aureus :* (*a*) 0·8. 0·7; (*b*) 1·4, 1·3; (*c*) 2·2, 1·8; (*d*) 5·0, 4·9; *B. diphtheriae :* (*a*) 2·3, 1·6; (*b*) 4·5, 3·5; (*c*) 7·3, 5·3; (*d*) 18·0, 14·0; *Streptococcus haemolyticus:* (*a*) 2·2, 1·7; (*b*) 4·4, 3·5; (*c*) 6·7, 5·3; (*d*) 16·0, 12·0; *Pneumococcus :* (*a*) 3·3, 3·0; (*b*) 6·6, 6·1; (*c*) 10·0, 9·4; (*d*) 23·5, 17·5. In the case of *B. tuberculosis*, determinations of the maximum dilutions which could kill the virulent organism in sputum in a 1 hour exposure were made and were as follows:—(*a*) 1: 20; (*b*) 1: 60; (*c*) 1: 80; (*d*) 1: 200. D. G. H.

Reduction of Methylene Blue in Milk. The Influence of Light. H. R. Whitehead. (*Biochem. J.*, 1930, **24**, 579–584.)—The fact that milk which contains living bacteria will decolorise methylene blue has been used for many years as a means for the determination of the degree of contamination of market milk supplies.

While using it for this purpose the observation was made that light has a significent effect on the progress of the decolorisation of methylene blue in milk. Samples of milk to which methylene blue had been added, incubated in a water-bath with glass sides, designed to facilitate observation of the tubes, gave unexpectedly short reduction times. Experiments were therefore devised to find an explanation of the mechanism of the process in milk. The results showed that methylene blue added to fresh milk of good quality is reduced in a short time in the presence of sunlight at 37° C. In darkness at 37° C. no decolorisation occurs within 7 hours. The reaction in sunlight is not due to an enzyme, for it proceeds equally well in milk which has been heated to 100° C. for 30 minutes. Milk from which the fat has been removed by centrifugal separation no longer gives the reaction, but the activity of the milk can be restored by an addition of sodium oleate. Sodium palmitate does not have a similar action. It is suggested that sunlight catalyses an oxidation-reduction reaction in which unsaturated fats are oxidised and methylene blue is reduced. The occasional return of colour in strong sunlight in the top layers of milk where fat is collecting may possibly be due to an autoxidation of the oxidised fats, resulting in a final oxidation-reduction potential sufficient partly to re-oxidise the methylene blue. Further work is projected in which the phenomenon will be examined in a more quantitative manner with the aid of a quartz mercury-vapour lamp, for it seems most probable that the active agent is ultraviolet light and that more powerful effects will be observed than with sunlight acting through one or two layers of glass. The phenomenon has obvious practical importance in connection with the keeping qualities of butter-fat in the presence of hydrogen acceptors. P. H. P.

Food Poisoning due to Eggs. W. M. Scott. (*Brit. Med. J.*, 1930, 56–58.) —During the past three years at least seven outbreaks of gastro-enteritis have apparently been caused by consumption of eggs, which in each instance were ducks' eggs. In a monograph on poisoning by whipped cream, prepared from cream, gelatin and white of egg, Lecoq (Paris, 1906) shows that the poisonous properties appear most commonly in summer and are due, not to metallic poisons or to putrefactive products such as putrefaction bases, tyrotoxin, etc., but to bacterial infection of the egg albumin. Lecoq's view is that infective material is most likely introduced into the oviduct of the bird during copulation, with infection of the albumin as it is being deposited round the yolk. Low (1913) suggested that infection of the ovary, oviduct, and egg with salmonella could take place as part of a general infection of the bird, such as is assumed with parrot subjects of psittacosis. The author has been unable to demonstrate this method of egg infection by direct experiment, but he has found that eggs, both of ducks and of hens, can become infected when immersed for a few days in cultures of *B. aertrycke*. If the culture is simply allowed to dry on, infection fails; part of the shell must remain moistened for penetration to occur. At first *B. aertrycke* multiplies slowly in the egg, but as the infected egg grows staler, multiplication becomes rapid and the egg represents an enormous dose, a loopful of either albumin or yolk yielding countless

colonies of the organism. Such an egg may appear no more than slightly stale, and would not be discarded by every cook. Not all eggs are easy to infect in this manner, and the risk of poisoning caused by consumption of eggs is comparatively remote, but it seems likely that attacks of gastro-enteritis due to this cause are more common than is generally supposed. T. H. P.

Toxicological.

Toxicity of Pyrethrum Vapours to Honey Bees. J. M. Ginsburg. (*J. Agric. Res.*, 1930, **40**, 1053–1057.)—Experiments in which honey bees were exposed to pyrethrum vapours for 48 hours were entirely negative, and it is concluded that the toxicity of ground pyrethrum flowers and of extracts is primarily due to the non-volatile substances, pyrethrine I and II, and not in any way to the essential oil. D. G. H.

Organic Analysis.

Analysis of Philippine Lumbang Oil. A. O. Cruz and A. P. West. (*Philippine J. Sci.*, 1930, **42**, 251–257.)—The lumbang or candlenut oil, cold pressed from the seeds, had the following characteristics : Sp. gr. at 29°/4° C., 0·9170; $n_D^{30°}$, 1·4740; saponification value, 191·7; iodine value (Wijs 6 hours), 152·7; free fatty acids 0·34 per cent., unsaponifiable matter, 0·34 per cent.; oxy-acids (by a modification of Fahrion's method), 0·13 per cent.; colour (Wesson colorimeter), 0·1 red, 1·5 yellow. The saturated and unsaturated acids were separated by the lead salt and ether method. An allowance was made for the small amount of oxidised acids (0·13 per cent.) contained in the saturated acids, and 2·02 per cent. of saturated acids were found. The composition of the unsaturated acids was determined by the bromine derivative method; alpha (14·65), beta (4·62), and delta (1·54) linolic acids made up 20·81 per cent. of the linolic acids which, subtracted from the total of 49·36 per cent. for linolic acid, left 28·55 for the gamma acid. The composition of this lumbang oil was:—Essential oil, 0·3; unsaponifiable matter, 0·3; glycerides of saturated acids, 2·1; oxidised acids, 0·1; linolenic, 7·7; linolic, 48·2; and oleic acid, 40·4 per cent. The differences in composition from those given previously (ANALYST, 1922, **46**, 27), *i.e.* higher per cent. of linolenic and linolic glycerides and lower per cent. of oleic glyceride, are accounted for by differences in experimental procedure. D. G. H.

Inorganic Analysis.

Separation and Determination of Titanium by Hydroxyquinoline. R. Berg and M. Teitelbaum. (*Z. anal. Chem.*, 1930, **81**, 1–8.)—Hydroxyquinoline produces in titanium solutions an orange-yellow crystalline precipitate, $TiO(C_9H_6ON)_2.2H_2O$: quantitative precipitation takes place in acetate or ammoniacal tartrate solution. The determination of titanium is made by ignition, or by drying at 110° C. to constant weight (Ti factor 0·1361), or bromometrically

(ANALYST, 1927, **52**, 431, 495); 1 c.c. of 0·1 *N* bromate-bromide solution = 0·000599 grm. Ti. The tartrate solution (150 c.c.) is treated with 0·5 grm. of sodium acetate, neutralised with ammonia against phenolphthalein, acidified with 2 c.c. of acetic acid, and warmed to 60° C. A 3 per cent. alcoholic solution of the reagent is added in excess (yellow colour of supernatant solution). The liquid is then boiled for 10 minutes. The precipitate is collected in a porous crucible and washed with hot water till the washings are colourless. If it is to be ignited it must be covered with about 3 grms. of anhydrous oxalic acid and heated gradually. For the volumetric determination, the washed precipitate is dissolved in strong hydrochloric acid and the solution titrated after dilution to 2 *N*.

Separation from alkaline earths and magnesia. The above directions may be followed. Alternatively, the feebly mineral acid solution is treated at 60° C. with an excess of precipitant and the liberated acid neutralised with sodium acetate. *Separation from aluminium.* The solution is treated with about 1 grm. of tartaric acid and 70 to 80 times as much malonic acid as will combine with the aluminium, and sodium acetate (about 1 grm.). After dilution to 150 c.c., neutralisation with ammonia against phenolphthalein, and acidification with 1 to 2 c.c. of acetic acid, the precipitation is carried out as directed above. The aluminium in the filtrate is determined by dropwise addition of a 3 per cent. hydroxyquinoline acetate solution and subsequent addition of a slight excess of ammonia at about 50° C. The liquid is digested at 80° C., and the precipitate treated as before (see ANALYST, 1927, **52**, 612). The aluminium precipitate dissolves slowly in strong hydrochloric acid, but rapidly in a 1:1 mixture of the acid and alcohol. The residual titration of the iodine with thiosulphate is carried out immediately after the bromate addition, hence bromination of the alcohol, which takes place only after more prolonged contact, need not be feared. W. R. S.

Determination of Free Hydrochloric Acid in Presence of Ferric and Aluminium Chlorides. K. Küchler. (*Chem. Ztg.*, 1930, **54**, 582.)—The solution is treated with a large excess of solid sodium (not potassium) fluoride (to form the difficultly soluble complexes Na_3AlF_6 and Na_3FeF_6) and well shaken; 5 grms. of solid sodium chloride are added (to reduce the solubility of the compound salts), and the liquid again shaken. Phenolphthalein is added not too sparingly, and the titration carried out, with continual shaking, to a permanent red colour. Should silicic acid occur in the liquid, there will be consumption of acid according to

$$6NaF + SiO_2 + 4HCl = Na_2SiF_6 + 4NaCl + 2H_2O,$$

but the red colour will vanish very quickly, as the fluosilicate is easily decomposed by the alkali. Correct results are obtained if the titration is carried to an end-point stable for several minutes. W. R. S.

Detection and Determination of Small Quantities of Lithium. E. R. Caley. (*J. Amer. Chem. Soc.*, 1930, **52**, 2754–2758.)—This precipitation process is based on the fact that lithium stearate is insoluble in amyl alcohol. A freshly-prepared 2 per cent. solution of ammonium stearate in amyl alcohol is used as the

precipitant. The amyl alcoholic solution of lithium chloride is treated with 2·5 times its volume of the precipitant. The lithium stearate is precipitated in a finely-divided form, yielding a more or less opaque suspension; hence it is proposed to determine small amounts by turbidimetry. It is essential that the sample and the standard should be treated in exactly the same manner as regards volume, mode of adding the reagent, and time of shaking. The author considers his process a useful supplement to Gooch's amyl alcohol process when the absolute amount of lithium is of the order of one mgrm., in which case the prescribed corrections in that process may exceed the amount of lithium present. W. R. S.

New Method for the Separation of Lithium from Potassium and Sodium. A. Sinka. (*Z. anal. Chem.*, 1930, **80**, 430–435.)—The separation is based on the solubility of lithium chloride and the insolubility of sodium and potassium chlorides in anhydrous dioxane, $O\langle\begin{smallmatrix}CH_2-CH_2\\CH_2-CH_2\end{smallmatrix}\rangle O$. The extraction of the mixed chlorides is carried out in a porous glass crucible suspended in a conical flask below the lower end of a reflux condenser. For fuller details the original paper should be consulted. W. R. S.

Microchemical.

Micro-Determination of Hydroxyl Groups. P. M. Marrian and G. F. Marrian. (*Biochem. J.*, 1930, **24**, 746–752.)—A micro-method for the determination of the percentage of hydroxyl in the higher fatty alcohols, based on the macro-method of Hibbert and Sudborough (*J. Chem. Soc.*, 1904, **85**, 933) is described; the method is based on the volumetric determination of the methane evolved when an alcohol reacts with Grignard's reagent. Certain precautions must be rigidly observed, or irregular results are obtained. It is necessary to work in an atmosphere of nitrogen and to avoid changes in temperature and pressure, for even small variations introduce appreciable errors. By suitable means the temperature can be kept sufficiently constant, but variations in barometric pressure have constantly caused trouble. The procedure, method of calculation and results obtained are described. Results accurate to ± 8 per cent. are obtained when the alcohol is readily soluble in the ether of the Grignard reagent used. The use of pyridine to dissolve alcohols which are not soluble in the ether of the reagent is not recommended, the results being very unreliable, owing to a variable blank. P. H. P.

New Method of Inorganic Gravimetric Micro Analysis. I. Determination of Small Quantities of Gold in the Presence of Large Amounts of Iron, Lead and Copper. J. Donau. (*Mikrochemie*, 1930, **8**, 257–263.)—Gold is precipitated quantitatively from dilute solutions on to small pieces of silk impregnated with hydrazine. The silk containing the gold is then washed, dried, ignited and weighed. *Detail*: Ordinary Japanese silk is prepared for use by

digesting it for a long time in hot dilute hydrochloric acid, and then washing with distilled water; the silk is then squeezed, dried, and kept free from dust. It is used in small squares of 0·1 cm.^{2} or 0·5 cm.^{2} area, and must only be touched with forceps. It should give no more residue on ashing than good filter paper. The reagent varies for the different substances to be determined; for gold the silk is placed for a short time in hot 50 per cent. hydrazine solution (the hydrochloride may be used), lightly washed a few times, pressed out and dried. The efficiency of the silk is tested by placing a 0·5 cm.^{2} piece in a few drops of a 0·1 per cent. solution of gold. The piece of silk turns purple or violet, but no reduced gold should form in the solution. When this occurs the silk should be re-washed and tested again. For the analysis the test solution is weighed out into a small glass vessel of about 0·5 c.c. volume, and heated on a heating block to 120° C.; a piece of treated silk about 0·5 cm.^{2} in area, is placed in the solution, and if it is not completely moistened, this must be done with a further drop of water. To precipitate 0·2 mgrm. of gold about 3 mgrms. of treated silk are required. A second piece of silk of the same size is added, and then a smaller piece, and so on, until no colour develops in the silk. The pieces of silk are then well washed by pouring on to a platinum micro filter (Donan, *Monatsh. Chem.*, **36**, 385), and washing, drop by drop, with hot water. The pieces of silk are lifted with platinum-tipped forceps into a small platinum boat, made of thin foil. The boat and the filter (which may contain a few threads of silk) are now dried at 120° C., and the boat is heated gently in the micro flame, and finally more strongly, until the silk is ashed. The filter is also held in the flame for a short time, with the forceps, until the threads are also ashed. Both are then heated to glowing on a quartz lid, and placed in a desiccator while hot. After 1 minute the filter and boat can be weighed. The difference in weights before and after the test gives the weight of gold. If the silk gives a weighable ash, the weight of silk added should be determined, and the calculated weight of its ash subtracted from the final weight. The whole determination takes 25 minutes. The determination is not affected by large amounts of other metals such as iron, copper or lead; but in the presence of other metals the silk should be more carefully washed. The amounts of gold determined varied from 0·05 mgrm. to 0·28 mgrm. Typical results obtained with a solution of pure gold containing 0·055 per cent. of gold, were 0·054, 0·055, 0·056 per cent.; in the presence of three to ten times the weight of iron, 0·053, 0·055 and 0·054 per cent.; in the presence of copper 0·056 per cent.; and in the presence of copper, lead and iron 0·055 and 0·054 per cent.

Any micro balance may be used, but the author used an improved model of the Nernst balance, to be described in his next paper. J. W. B.

Specific Test for Cadmium. F. Pavelka and E. Kolmer. (*Mikrochemie*, 1930, **8**, 277–280.)—The reagent used is nitrophenyl-arsenic acid, which gives with the cadmium ion in acetic acid solution a white to yellow crystalline precipitate; under a 200 times magnification the crystals appear as long narrow rectangles. For amounts of cadmium greater than 10γ the test is carried out in

pointed micro test tubes, in 10 per cent. acetic acid; the reagent is added in aqueous solution, and, if necessary, the solution is heated over the water bath. The limit of concentration is 1:200,000, and the smallest amount identifiable is 5γ. When carried out on a slide, with the use of a microscope, the limit of concentration is 1:10,000, and the smallest amount identifiable $0{\cdot}1\gamma$. The reaction takes place in concentrations of acetic acid up to 25 per cent., but the precipitate is soluble in concentrated acetic acid and mineral acids, or in dilute mineral acids on heating, but is reprecipitated by adding sodium acetate, if the resulting acidity is that of 20–25 per cent. of acetic acid or a *p*H of at least 2·2. This reaction is specific for cadmium, as other metals do not react in acetic acid solution. Amounts of 5γ of cadmium can be identified in the presence of 1000 times the amount of zinc, lead or mercury, and 100 times the amount of bismuth. J. W. B.

Physical Methods, Apparatus, etc.

Spectrographic Analysis of the Various Fluorescences of Olive Oil under Ultra-violet Radiation. H. Marcelet and H. Debono. (*Compt. rend.*, 1930, **190**, 1552–1553.)—Spectrograms on ordinary and on super-sensitised micro-panchromatic plates have been prepared for Tunisian, Algerian, and Moroccan olive oils subjected to filtered ultra-violet radiation, which produces mauve, yellow, apricot, green, whitish or chocolate fluorescence, showing no correspondence with the natural colour of the oil. With all the oils, the emission spectra exhibit between 5700 and 6900 Å.U., a luminous field having, for certain oils, a maximum intensity between 6650 and 6900 Å.U. corresponding with the ray regarded as characteristic for olive oil; this ray is observed only with oils showing mauve, brick-red or apricot fluorescence. The luminous field extends continuously from 5200 to 6900 Å.U. with oils exhibiting green fluorescence, and from 4900 to 5500 Å.U. and then from 5700 to 6900 Å.U. with other oils. As regards absorption spectra, all the oils show two bands at 4380–4750 and 5640–6900 Å.U., oils with mauve, apricot or whitish fluorescence giving also a more luminous zone at 6650–6900 Å.U. Moreover, oils displaying apricot or yellow fluorescence present a band at 3825–4280 Å.U., and those with whitish fluorescence one at 5000–5640 Å.U. The slight differences between the spectrograms corresponding with different fluorescences are not sufficiently marked to modify sensibly the spectrum of olive oil. T. H. P.

Reviews.

AN INTRODUCTION TO SURFACE CHEMISTRY. By ERIC KEIGHTLEY RIDEAL, D.Sc., F.R.S. 2nd Edn. Pp. vi+459. Cambridge: University Press. 1930. Price 21s.

The first edition of Professor Rideal's book (1926) was reviewed in THE ANALYST, 1926, 51, 598. The second edition has been extended by 123 pages, and "an attempt has been made to revise and review our present knowledge of the properties of interfacial phases."

We must agree with the author that in investigations—academic and technical—of the chemistry and physics of colloidal systems, "as a preliminary to such investigations an adequate understanding of the properties of the simpler systems such as surfaces and interfaces has become not less, but rather more, important as the complexity of the subject increases."

The nine chapters treat successively of:—The Surface Tension of Liquids; The Surface Tension of Solutions; The Surface Films of Insoluble Materials; Liquid-Liquid Interfaces; The Gas-Solid Interface; The Liquid-Solid Interface; Differences of Potential at Interfaces; Conditions of Stability in Suspensions and Emulsions; Gels and Hydrated Colloids.

Professor Rideal is an active investigator in all the above-mentioned fields of colloid chemistry and physics, and his book assumes an authority which merits attention. To all interested in the modern development of surface and colloid phenomena, and especially to the advanced student of colloid physics, Professor Rideal's book is most warmly commended. The printing and binding are excellent and the price is very reasonable.

WILLIAM CLAYTON.

LATEX, ITS OCCURRENCE, COLLECTION, PROPERTIES AND TECHNICAL APPLICATIONS. By ERNST A. HAUSER, Ph.D., with Patent Review compiled by CARL VON BORNEGG, Ph.D. Translated by W. J. KELLY, Ph.D. Pp. 191, 73 illustrations. Published by The Chemical Catalog Co., Inc., New York. Price $4.

This book is a translation of the original German edition of 1927. As the only book devoted entirely to latex it should be of considerable value to rubber chemists, but the reviewer feels that it cannot be regarded as a thorough-going monograph. For example, no advantage has been taken of the opportunity to bring the book up to date as regards certain of the applications of latex. In particular, dipping processes, which represent an application of latex actually in commercial operation, would seem to deserve more thorough treatment than a

single page in Chapter XIII. Similarly, the patent literature still terminates at 1927. On the whole, however, the book is comprehensive.

Following an excellent historical introduction, there are descriptions of latex-bearing trees and of the collection and properties of latex. Naturally *Hevea* latex is primarily discussed, but the descriptions accorded to the latices of gutta and balata, etc., are especially valuable owing to the scant reference to these in the previous literature. Subsequent chapters are devoted to the coagulation, preservation, transportation, concentration, vulcanisation and applications of latex. These are quite satisfactory, as far as they go. The book concludes with a chapter on the internal structure of rubber, which is a good review of our present knowledge of this subject. The close connection between the structure of rubber in the mass and the structure of the latex particle itself, on which the author is a well-known authority, is the reason for the introduction of a subject otherwise outside the scope of the work.

The book is well printed and illustrated, although the lettering to the figures on p. 138 appears to have been omitted. The constant reiteration of the word "proven" will irritate English readers. The book bristles with references, but the usual practice of giving the volume number in heavy type has not always been adhered to. The omission of a list of abbreviations is noted. The book may be recommended to the general reader, although the price may be considered high.

W. H. STEVENS.

A TEXT-BOOK OF INORGANIC PHARMACEUTICAL CHEMISTRY FOR STUDENTS OF PHARMACY AND PHARMACISTS. By CHARLES H. ROGERS, D.Sc. in Pharm. Pp. 676. Philadelphia: Lea and Febiger. Price $7.0.

The title of this book is somewhat misleading. It is not confined to pharmaceutical chemistry, but is a text-book of Inorganic Chemistry on more or less conventional lines, with additional information on the pharmaceutical preparations and uses and on the pharmacological actions of the substances described. The author does not stray far beyond the official preparations of the United States Pharmacopoeia and National Formulary, and therefore the book has a very limited interest for pharmacists or students in this country.

The material is not confined to inorganic chemicals, the majority of organic salts of inorganic bases being included, but there are some important omissions, *e.g.* the bismuth tartrates and sodium antimonyl tartrate. Other omissions noted in passing are the absence of any pharmaceutical use for kieselguhr or adsorptive carbons. In fact, the greater part of the pharmaceutical information given can be obtained from the official works mentioned above. Incidentally it gives one a slight shock to see *Liquor Pancreatini* and *Syrupus Zingiberis* included as pharmaceutical preparations of magnesium carbonate, until one realises that the latter is used as a clarifying agent in their preparation.

Methods of analysis are practically confined to the quantitative determination of the chemicals described, and here, again, official and conventional methods are

described. There seems to be little appreciation of the limits of accuracy of analytical methods—or indeed of atomic weight determinations. We are told that 1 c.c. of $N/10$ permanganate is equivalent to 0·00204266 grm. of potassium chlorate, and this in a method for which 0·1 grm. of potassium chlorate is weighed out. In the methods described the usual United States Pharmacopoeia practice of drying the sample before analysis is copied, but it does seem to be carrying this principle rather far when it is directed to ignite a sample of calcium oxide to constant weight before weighing out a quantity for analysis.

The chemical information, too, is not always accurate. Both calcium and sodium glycerophosphates are described, but no mention is made of the isomeric α- and β- modifications. The formula given for the calcium salt is the α variety whilst the formula of the sodium salt is the β variety, but the method of preparation given for the latter would produce chiefly the α salt. Precipitated calcium phosphate is described as nearly pure $Ca_3(PO_4)_2$, whereas it always contains a large proportion of the dibasic salt.

Misprints are few—there is an amusing one on p. 335, where it is stated that "Gypsum is used as a filter for wall-paper"—but there are numerous slipshod and inaccurate statements, of which the following may be quoted. "Thyroxin contains *not less than* 63 per cent. of iodine." "Iodine combines with milk to form non-toxic *iodides.*" Solutions of dialysed iron "contain about 98·5 per cent. of colloidal ferric hydroxide and about 1·5 per cent. of ferric chloride." "Reduce the acidity of the solution *to a minimum* with sodium carbonate."

The book is well printed and illustrated, and it is a pity that, considering the large amount of work which has gone to its preparation, it should be marred by the defects which have been mentioned.

NORMAN EVERS.

ATLAS DER BLEISTIFTSCHRIFT. By SIEGFRIED TÜRKEL. Pp. 48, with 76 plates. Graz: Ulr. Mosers Buchhandlung. Price fl. 25.63.

This book, as its title suggests, is not an ordinary text-book, but consists of a collection of photographs and photomicrographs of pencil markings, with a few pages of explanatory text. The first fourteen plates show the apparatus (microscopes, cameras, lamps, etc.) which the author has found most suitable for obtaining photographic records by direct and transmitted light of graphite marks, and the remaining plates give examples of the results which can be obtained by varying the methods.

In his descriptive text Professor Türkel deals briefly with the preparation of blacklead pencils, their chemical composition, and the chemical characteristics of the material on the paper. The tests devised by the reviewer are given in detail, and the author rightly lays stress upon the necessity for making blank tests upon the paper itself. Incidentally he mentions that the paper may contain traces of titanium, but, in the reviewer's experience, such traces are insufficient to give the yellow coloration with hydrogen peroxide and hydrochloric acid, and any pencil

giving that reaction must be looked upon as a very distinctive specimen. It is interesting to note that Professor Emich has made experiments to ascertain whether it is possible to distinguish between pencil marks by the ordinary micro-chemical methods, and has found that about 2 mgrms. of the substance would be required—an amount which would hardly be obtainable from an ordinary document.

This "Atlas" shows what can be done by optical methods in criminological work, and may be recommended as a very valuable contribution to a difficult branch of the subject.

EDITOR.

Publications Received.

A COMPREHENSIVE TREATISE ON INORGANIC AND THEORETICAL CHEMISTRY. Vol. X. By J. W. MELLOR. London: Longmans, Green & Co. Price 63s. net.

THE SPIRIT OF CHEMISTRY. By A. FINDLAY. London: Longmans, Green & Co. Price 10s. 6d. net.

CHEMICAL METHODS IN CLINICAL MEDICINE. By G. A HARRISON. London: J. & A. Churchill Price 18s.

THE MICROBIOLOGY OF STARCH AND SUGARS. By A. C. THAYSEN and L. B. GALLOWAY. Oxford University Press. Price 25s. net.

COLLOIDS: A TEXTBOOK. By H. R. KRUYT. Second Edition. New York: Wiley. London: Chapman & Hall. Price 17s. 6d.

THE SPONTANEOUS COMBUSTION OF HAY. By C. A. BROWNE. Tech. Bull. No. 141. U.S.A. Dept. Agriculture. Price 10 cents.

REDUCTION OF DATA ON MIXTURE OF COLOUR STIMULI. By D. B. JUDD. Research Paper No. 163. U.S.A. Dept. of Commerce. Bureau of Standards. Price 10 cents.

SPECIFIC GRAVITY AND BAUMÉ GRAVITY TABLES FOR TURPENTINE. By W. P. SMITH and F. P. VEITCH. U.S.A. Dept. Agriculture. Circular No. 110. Price 5 cents.