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The Effect of Giving Certain Oils in the Daily Diet of Cows on the Composition of Butter Fat.

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THE work described in this paper is the result of the second part of an experiment planned with two objects in view:

(a) To obtain further and more exact information on the effect of cod-liver oil feeding of cows on the vitamin *A* content of the milk, than had been obtained in a similar experiment reported by Drummond, Coward, Golding, Mackintosh and Zilva (1923).

(b) To attempt to make a quantitative study of the composition of butter fat and the alteration in its composition induced by the inclusion of various oils in the daily diet of the cows.

The first part of the experiment on the vitamin content of butter is being reported in the Journal of Agricultural Science, 1924, but, owing to the fact that the conditions obtaining in the experiment were governed by the two objects in view, it will be necessary to make some reference to it in this paper. The effect of the administration of oil-cakes, oils and various special foods has been much studied, the method usually adopted being to follow the alterations in the composition of the butter fat by a determination of the usual analytical values of samples of butter fat from the cows under experiment. Further, iodised fats, or oils giving specific colour reactions, have been given in the diets of cows, and tests for iodine, or the colour reactions concerned, carried out on the butter-fat. This type of experiment is open to the objection that, even if iodine

311

were found present in the butter fat, or the colour reaction found positive, it is still uncertain whether the oil itself, or the substance giving the reaction only, has passed into the butter fat; in the case of iodised fats, the presence of iodine in the butter fat does not necessarily mean that the iodine is still attached to the fat or other substance which was given in the food. Although much work of this nature has been carried out, very little quantitative work has been attempted, and, in view of its importance from the physiological point of view, it seemed worth while attempting to determine what fatty acids were passed into the milk and under what conditions.

The general plan of the experiment was to give to cows which were under strictly controlled conditions, a basal diet for a sufficiently long period to cause the butter fat to be of something approaching constant composition; at the end of this period, various oils were added to the diet, and after a further period on the basal diet when no oil was given, another oil was administered; after which the cows were put to pasture. The basal diet chosen was one which was as free from fat as possible, in order that the effect of the administration of the oils at a later date on the composition of the butter fat might be the more marked. At intervals samples of butter made from the milk of each cow were examined for the usual analytical values: it was planned to convert the acids of the various butter fats into their methyl esters and to attempt a quantitative separation by a fractional distillation of these esters after the end of the experiment.

PARTICULARS OF THE COWS USED.—The cows chosen for the experiment were dairy shorthorns selected from the herd of the National Institute for Research in Dairying at Reading.

TABLE I.

Cow.	No. of calf.	Last calved.	Condition.
Lily ..	1	17/11/22	Fairly good.
Lucy ..	1	4/12/22	Good.
Scarlet ..	3	8/11/22	Good.
Fillpail II. ...	7	5/1/23	Fairly good.

The cows, all of which had recently calved, were placed in well-lighted stalls at various times between the middle of December and the beginning of January, and were given the basal diet up to February 26th.

COMPOSITION OF THE BASAL DIET.—The basal diet consisted of mangolds, hay and concentrates, the latter made up of equal parts of maize gluten, crushed wheat and soya bean meal, the last having had its fat commercially extracted. The daily ration of each cow varied little from the following:—Mangolds, 60 lbs.; hay, 15 lbs.; concentrates, 9 lbs. (Full details are given in Table 2.)

FAT INTAKE ON THE BASAL DIET.—The percentage of ether-soluble matter in the dried foods was found to be:—Mangold, 0·1; hay, 2·2; maize gluten, 2·2; soya bean, 1·2; and crushed wheat, 1·7 per cent. (dry substance). A half day's ration, after careful drying and powdering, was extracted to remove fat, and

yielded 66 grms. of a heavy green oil, having a saponification value of 169·5, iodine value 118·4 and containing 18·75 per cent. of unsaponifiable matter. A table is given later in which the average amount of ether-soluble matter, which can be regarded as saponifiable glycerides, received daily by each cow and worked out over weekly averages for each period, is given.

Throughout the experiment until the final period of grass-feeding, the cows were kept in stall, being only allowed out for a period of fifteen minutes every morning into a clean yard. Records of milk-yield were made at both morning and evening milkings, and samples of every milk were submitted for determination of the percentages of fat and total solids and specific gravity. The food consumption of each cow was measured daily, and close observations of the general conditions of the animals were maintained.

At intervals during the experiment, samples of butter were examined for the usual analytical values and the vitamin concentration, and were then stored at 0° C. in the dark for the quantitative work which it was hoped to carry out at a later stage. Care was taken that samples were always taken at the beginning and end of each period, and intermediately.

PROGRESS OF THE EXPERIMENT.—The duration of the experiment can be conveniently divided into five periods.

Period.	Date.	Diet.
1	12/12/22–26/2/23	Basal: Concentrates, roots, meadow hay.
2	27/2/23 –14/3/23	Basal diet as above +daily doses of coconut or arachis oil.
3	15/2/23 –16/4/23	Basal diet: Concentrates, roots, seed hay.
4	17/4/23 –17/5/23	Basal diet +cod-liver oil.
5	18/5/23 –to end.	Pasture.

Unfortunately, one of the cows, Fillpail II., died suddenly in the early part of period 2. Careful post-mortem examination failed to reveal the cause of death; the other cow receiving coconut oil went off her feed, but her appetite returned rapidly on the cessation of giving this oil.

METHOD OF ADMINISTERING THE OILS.—The oils were given in amounts beginning at 2 ozs. per day and rising to 8 ozs. in small increments, as the cows became accustomed to them. During the oil periods the total weight in lbs. of oil given to each cow was:

	PERIOD 2.			PERIOD 4.		
Oil.	Lily	Lucy	Scarlet	Lily.	Lucy.	Scarlet.
Arachis	9·5	9·5	—	—	—	—
Coconut	—	—	9·5	—	—	—
Cod-liver	—	—	—	10·75	10·75	10·75

The oils were given at the morning feed at first, and later, when larger doses were being given, in two portions at morning and evening feeds. They were mixed with the concentrates and were readily taken by the cows.
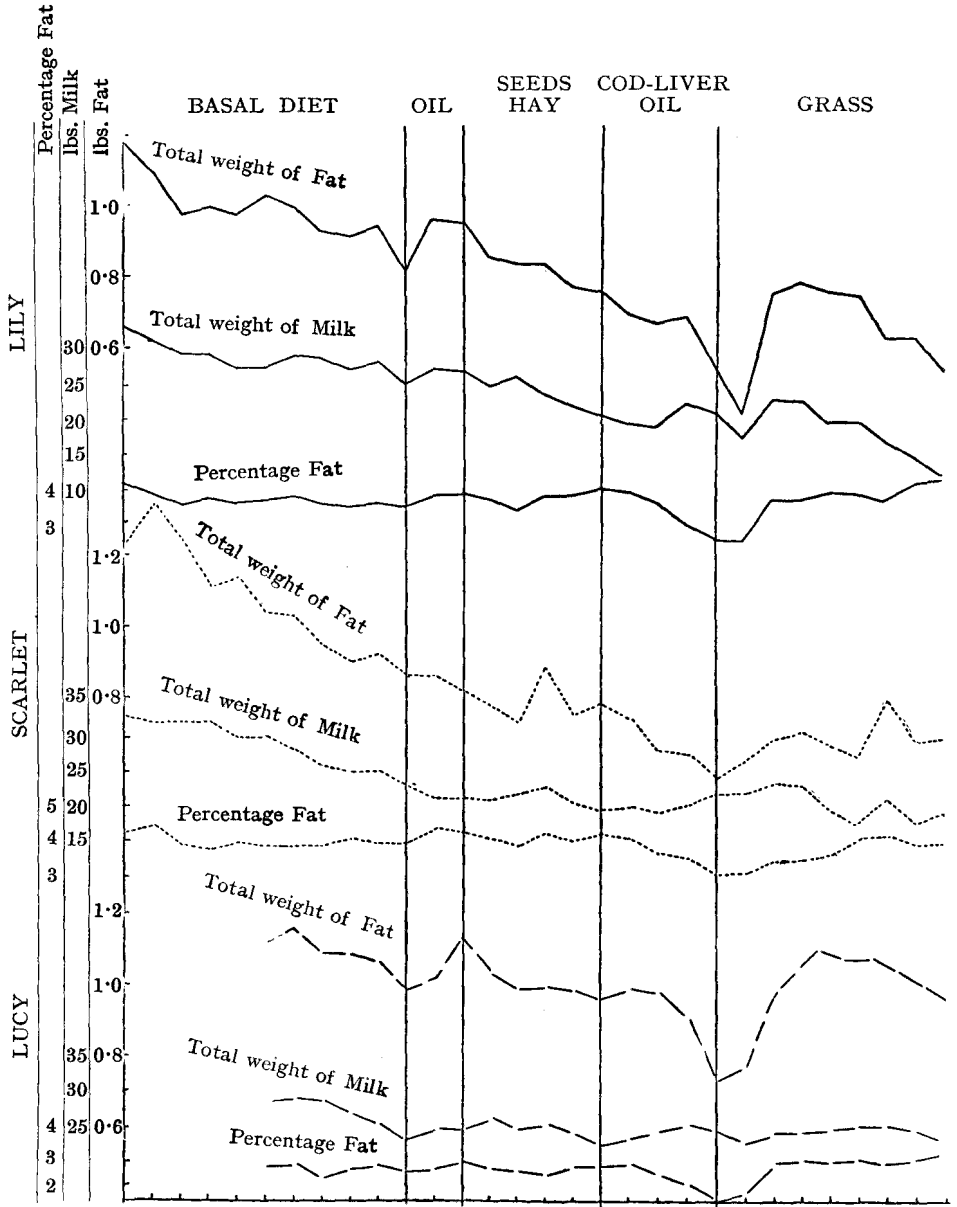
FIG. 1.   ←— Four Weeks —→

The food consumption of the animals was satisfactory throughout the experiment. Such small variations were encountered that the average figures for each period may be accepted as representing the daily intake.

TABLE 2.

Average daily diet in lbs. during the various periods.

Period.	Date.	Diet.	Lily.	Lucy.	Scarlet.
1	12/12/22–26/2/23	Mangolds	52·7	51·6	50·5
		Meadow hay	15·9	15·7	16
		Concentrates	9·1	11·1	11·2
2	27/2/23 –14/3/23	Mangolds	60	58	58
		Hay	15	15	15
		Concentrates (and arachis or coconut oil)	9	12	8·4
3	15/3/23 –16/4/23	Mangolds	60	60	60
		Hay	15	15	15
		Concentrates	7·6	12	8·3
4	17/4/23 –17/5/23	Mangolds	60	60	60
		Hay	15	15	15
		Concentrates (+cod-liver oil)	8	12	8
5	18/5/23 –	Pasture.			

In the final period all three cows were let out to pasture, which was in very good condition; the administration of cod-liver oil was discontinued, except in the case of Scarlet, which continued to receive ½ lb. of oil per day for a time.

RESULTS:—1. YIELD OF MILK AND MILK FAT. Daily records of these values were carefully taken, but for the purpose of simplifying the charts by eliminating minor fluctuations, we give, in Fig. 1, the average daily weight of milk and of fat and also of the percentage of fat in the milk for each cow over periods of one week. In general, it may be said that the curves given in Fig. 1, resemble the lactation curves of normal animals, but there is, in the case of all three cows, a definite fall in the percentage fat during the period in which supplements of cod-liver oil were given. This fall was promptly made good as soon as the cows were put out to pasture in period 5, even in the case of Scarlet, which received cod-liver oil for a time after she had been put out to grass. We are not prepared at this stage of our enquiries to give an opinion on the fall in fat content of the milk during the cod-liver oil period (it would appear to be of an order definitely greater than that which may occur during the late stages of lactation) and further experiments will be necessary to determine whether cod-liver oil in relatively large doses can exert an inhibitive action on the amount of milk-fat formed. No such effect was noted in last year's experiment already reported (Drummond, Coward, Zilva, Golding and Mackintosh, 1923), but in these cases smaller doses of ½ to 4 ozs. of oil were administered. It will be recalled that in Hopkins' experiment on goats (1920) the supplementing of a ration of mixed cereals, hay and

mangolds with green food, caused a bigger secretion of milk, but did not influence the proportion of total solids or fat.

The following table shows the average daily weight of fat in the milk per day, the percentage of fat and the fat ingested by the cows in the food over the five periods, but it may be unwise to lay too much importance on figures which are averaged over periods differing considerably in length.

	Lily.			Lucy.			Scarlet.		
Period.	Fat in Milk. lbs. per day.	Fat in Food. lbs. per day.	Fat in Milk. Per Cent.	Fat in Milk. lbs. per day.	Fat in Food. lbs. per day.	Fat in Milk. Per Cent.	Fat in Milk. lbs. per day.	Fat in Foods. lbs. per day.	Fat in. Milk. Per Cent..
1	0·95	0·37	3·7	1·07	0·39	3·9	1·08	0·39	3·7
2	0·95	0·95 (arachis oil)	3·8	0·84	0·94 (arachis oil)	4·2	1·06	0·98 (coconut oil)	3·8
3	0·80	0·34	3·7	0·78	0·35	4·0	0·99	0·39	3·6
4	0·60	0·69 (cod-liver oil)	3·3	0·65	0·60 (cod-liver oil)	3·5	0·86	0·74 (cod-liver oil)	3·3
5	0·68	—	3·7	0·69	—	3·6	1·08	—	3·8

It will be seen that the fall in the percentage and total fat is distinctly more marked in period 4 than in any other period.

2. QUALITY OF THE MILKS AND BUTTERS.—All the samples of milk from which butters were made were carefully observed during churning. No sample of milk was found to possess a "fishy" flavour, but the considerable abnormalities which were noticed in certain samples during butter-making are dealt with in the sections on the chemical analysis of the butter-fats. During the periods of oil-feeding, certain of the butters tended to be a little oily, but this was chiefly when the animals were retaining the vegetable oils in period 2. In period 4, no "taint" was noticed in the butters, even when the cows were receiving as much as ½ lb. of cod-liver oil per day each.

3. VITAMIN CONTENT OF THE BUTTERS. This question forms the subject of another examination (*J. Agric. Sci.* 1924). The results may be summarised as follows:—

(a) The typical winter ration of concentrates, roots and hay, may be adequate to maintain the vitamin *A* value of the milk-fat for considerable periods of time, provided that at least one of its components supplies adequate amounts of that dietary principle. Fresh green meadow hays are in this respect greatly superior to dry, brown, seeds hays.

(b) The addition of cod-liver oil to a winter ration deficient in vitamin *A* will induce a sharp rise in the vitamin *A* concentration of milk-fat. No such effect is seen when oils deficient in this dietary accessory (coconut and arachis oils) are given.

4.   CHEMICAL ANALYSIS.—

(a)   Reichert-Wollny Values.

| Period.	Date.	Diet.	Lily.	Lucy.	Scarlet.
1	11/12/22	Basal	34·4	—	—·
	18/12/22		31·6	—	30·2
	23/1/23		32·9	31·2	28·4
	19/2/23		29·8	28·7	27·5
2	5/3/23	Basal oil (Scarlet, coconut; Lily, Lucy, arachis)	31·5	32·2	28·5
	15/3/23		29·6	30·9	25·2
3	9/4/23	Basal (seeds hay)	29·3	31·2	29·4
4	30/4/23	Basal (cod-liver oil)	30·0	31·4	30·2
	13/5/23		24·9	23·5	26·5
5	29/5/23	At grass, Scarlet received cod-liver oil until	23·8	20·9	21·9
	19/6/23		24·1	23·0	23·4
	9/7/23		20·1	21·5	21·5

FIG. 2.   REICHERT-WOLLNY VALUE.

LILY
SCARLET
LUCY
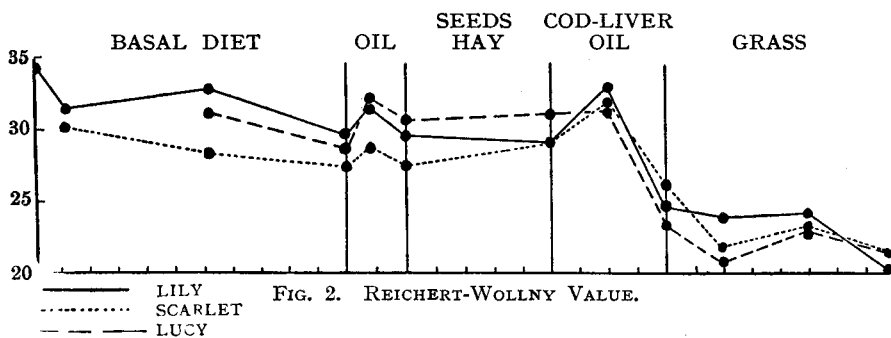
Fig. 2 gives the fluctuations of the Reichert-Wollny value of the butter fats from each individual cow throughout the experiment.   The average value for normal butter fat is 28·5; for coconut oil, 8·5; cod-liver oil and arachis oil do not contain water-soluble volatile acids.   The curves show a general fall in the Reichert-Wollny values throughout the seven months of the experiment.   It will be observed that the values were higher at the beginning than are the average values, and this is to be expected, as the cows had calved just prior to the commencement of the experiment.   There is no effect apparent when the oils were given in period 2, at the end of which the values differ but little from those at the beginning, and the general level is maintained at 30 through the third period, when no oil was given.   It is to be expected that a decided diminution in the amount of volatile soluble acids would occur in period 4 when cod-liver oil was given for 4½ weeks.   This is borne out by curves which show marked similarity of slope; at the end of this period the average value was below the limit of 24, which is usually taken for unadulterated butter fat.   It is curious to note that the Reichert-Wollny values tended to rise in the middle of the cod-liver oil period,

and this seems to indicate that there are unknown factors in this type of experiment which are uncontrolled. Further, the fall in the values during this period from 30 to 23 was continued for a time after the cows were put out to grass, although it was less rapid at this stage. There was no real recovery in the amount of volatile acids in the butter fat, even after the cows had been out at grass for 8 weeks. This may suggest that the fall during the cod-liver oil period was due to the advancement of the lactation period. It was shown by Nilson for Swedish butter that the Reichert-Wollny value of the butter fat from a group of cows fell from 33·4 in the first month of lactation to 25·4 in the fourteenth month. Many other workers report the big fall in the volatile acids at the end of the lactation period, but it is clear that the fall in our experiment from 33 in the first month to 22 in the seventh is of considerably greater magnitude, and is presumably due to the administration of an oil devoid of those acids. This is confirmed by the other analytical data.

(b)   Saponification Values.

Period.	Lily.	Lucy.	Scarlet.
1	232	—	—
	227·7	—	232
	234·3	230·5	238·5
	236·7	228·8	228·8
2	231·6	234·3	235·6
	222·2	229·3	229
3	237	238·4	235·8
4	233·8	229·2	229·2
	221·9	219·5	222·5
5	219·0	217·3	216·4
	218·9	216·8	217·3
	218·1	219	220·3

FIG. 3.   SAPONIFICATION VALUE.

——— LILY
·········· SCARLET
— ·· —— LUCY
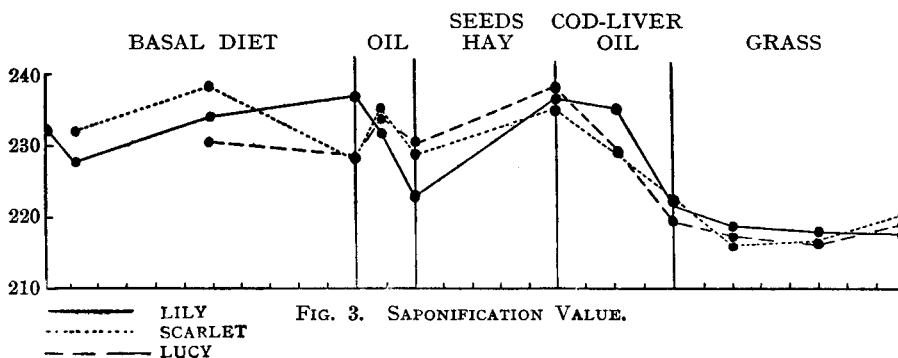
The average saponification value of normal butter fat may be taken as 227; of coconut oil, 257; arachis oil, 193; cod-liver oil, 185. Fig. 3 shews that the saponification values at the beginning of the experiment were higher than normal, following the Reichert-Wollny value. In the case of one cow only is there an appreciable fall in the saponification value of the butter fat at the end of the

arachis oil feeding, which is in agreement with the results obtained from the iodine values.   There is no rise in the value from the butter fat of the cow receiving coconut oil at the end of the period, but a distinct rise in the middle, which is reflected in the rise in iodine value and refractive index of the butter concerned. An average rise of about 7 in the saponification values had occurred at the end of period 3 during which the meadow hay of the diet had been replaced by seeds hay and when no oil was being given.   Immediately following the administration of cod-liver oil in period 4, the values begin to fall, and at the end of this period, 4½ weeks later, they have reached the low figure of 221.   There is no marked rise in the values when the cows return to grass, but at the end of eight weeks they are tending to increase.   This failure to return to normal values is also seen in the Reichert-Wollny and iodine values, and the refractive index.

(c) *Iodine Values.*

Period.	Lily.	Lucy.	Scarlet.
1	38·6	—	—
	40·3	—	39·7
	31·8	25·5	30·1
	28·7	32·2	31·0
2	36·4	33·2	27·4
	39·9	40·8	33·4
3	28·0	25·6	27·0
4	41·8	38·6	35·0
	55·6	52·1	46·6
5	46·7	46·8	51·0
	45·3	45·2	44·5
	47·1	46·3	46·9

The average iodine value for normal butter fat is 33; of coconut oil 8·5, and of arachis and cod-liver oils 90 and 170 respectively; hence the effect to be anticipated, it direct passage of the fatty acids occurs, is a decrease in the values of the butters from cows fed on coconut oil and a corresponding increase in those from cows fed on arachis oil; cod-liver oil should yield butters shewing abnormally high iodine values.   Fig. 4 gives curves shewing the variations in iodine values throughout the experiment.   In one case only of the cows receiving arachis oil is there a distinct rise, while the butter from the cow receiving coconut oil had an iodine value at the end of the period as high as that at the beginning.   This curious result is confirmed by the refractive indices which are given in Fig. 5. The iodine values show a slight fall during the period when the cows were receiving seeds hay in the basal diet, but from the commencement of the cod-liver oil period there is a sharp rise, which is maintained throughout, from an average figure of 26·9 at the commencement to 51·4 at the end.   There was a general fall in the values after the cows were turned out to pasture, save that the iodine value of the butter fat from Scarlet, which received further cod-liver oil for a period while at grass, shows a further rise until administration of the oil ceased.   It will be observed that even at the end of the 8 weeks' grass-feeding period the iodine values tend to remain high.

FIG. 4.   IODINE VALUE.

———— LILY
·········· SCARLET
— — —— LUCY

FIG. 5.   REFRACTIVE INDEX AT 40°C.
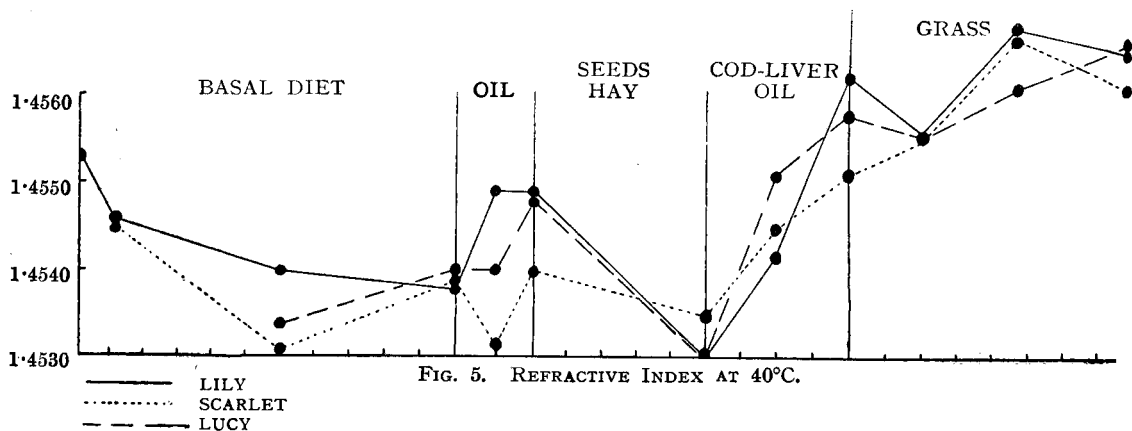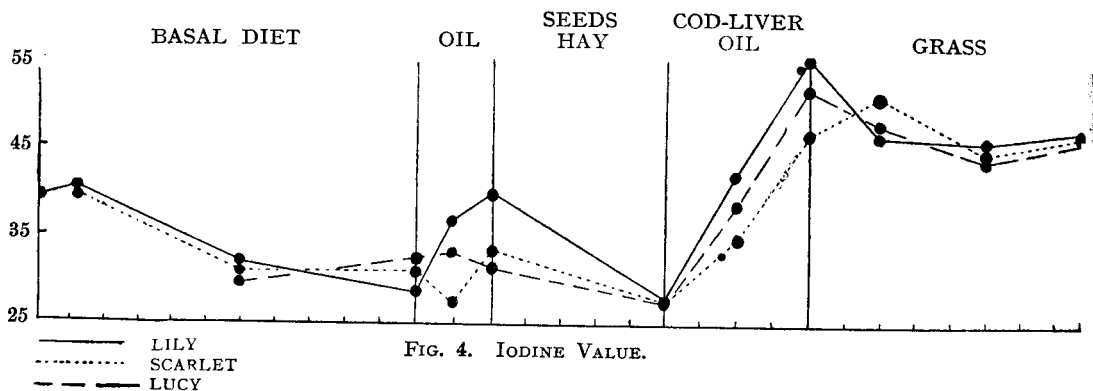
———— LILY
·········· SCARLET
— — —— LUCY

(d)   *Refractive Index.*   (40° C.)

Period.	Lily.	Lucy.	Scarlet.
1	1·4553	—	—
	1·4545	—	1·4545
	40	1·4534	31
	38	40	39
2	49	40	32
	49	48	40
3	31	30	35
4	42	51	45
	62	58	51
5	56	56	55
	61	68	67
	65	66	61

The refractive index of normal butter fat is 1·4548; of coconut oil, 1·4493; arachis oil, 1·4745; cod-liver oil, 1·4723.   In general, the refractive index follows the same course as degree of unsaturation, and hence there should be a similarity between the curves shewing refractive indices and iodine values.   The curves of Fig. 5 resemble those of Fig. 4.   There is a rise from an average of 1·4540 to

1·4548 in the refractive indices of the butters from the cows receiving arachis oil; but a slightly higher value than that of the butter at the beginning of the period obtains in that from the cow receiving coconut oil.   There is a decided fall in all the values in period 3 to about 1·4535.   The administration of cod-liver oil caused a marked rise which continued throughout the period to a value 1·4556.   A temporary fall then occurred after the return of the cows to grass, but Scarlet, which was still receiving oil, yielded a butter of still higher refractive index.   This temporary fall is followed by a rise until the end of the experiment, although by this time the iodine values had fallen somewhat.

Some explanation of this may be found in the fact that as the amount of water-soluble volatile acids decreases as the period of lactation progresses, the refractive index tends to rise.   Against this is the fact that increase in iodine value causes an increase in refractive index, which is much more marked than the effect due to the variation in the Reichert-Wollny values.   These latter had fallen to the low figure of 22·2 from 24·9, and this would therefore cause a rise in the refractive index, though the rise would not be expected to be such as to counterbalance the fall caused by the decrease of the iodine values from 51·4 to 46·7.
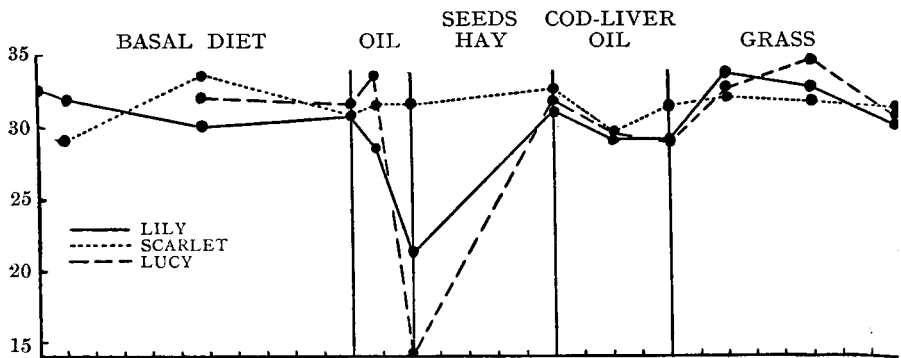

FIG. 6.   MELTING POINT.

Melting Point.

Period.	Lily. °C.	Lucy. °C.	Scarlet. °C.
1	32·5	—	—
	31·5	—	29·0
	30·0	32·0	33·0
	30·5	31·5	30·5
2	28·5	33·5	31·5
	21·0	14·0	31·5
3	31	31·5	32·5
4	29	29·5	29·5
	28·7	28·7	31·2
5	33·5	32·5	32·0
	32·5	34·5	31·5
	30·0	30·0	31·0

The melting point of butter fat is 29° to 33° C.   Very abnormal melting points were found in the butter from the cows receiving arachis oil, falling as low

as 14·5° C. This result is curious, for the other analytical values obtained, though abnormal, do not suggest so great an alteration in the composition of the butter fat. That abnormal effects on melting points can be obtained is shewn by the fact that the use of cotton seed cake is reputed to cause a rise in melting point. The use of cod-liver oil in our experiment had very little effect, even though the cows received 10·75 lbs. each in the course of 4½ weeks; the coconut oil did not cause any appreciable alteration in the melting point of the butter.

DISCUSSION.—Consideration of the curves indicates that no very marked effect, apart from the abnormally low melting points just referred to, on the characteristics of the butter fat resulted during the period when the basal diet was supplemented by coconut and arachis oils. This is a little surprising, in view of the definite change induced by the cod-liver oil feeding, although the period during which cod-liver oil was being given was more than twice as long as that of the other oils. It was to be expected that even though the effect due to coconut oil, which bears some similarity in composition to butter fat, was not marked, more definite evidence would have been obtained from the administration of arachis oil, which gives analytical values resembling those of cod-liver oil.

During the cod-liver oil period the analytical values all varied markedly in the direction indicating considerable passage of the oil or of some of its constituents into the milk. The slopes of the curves of all the values are very similar, and the close resemblance over the whole experiment is striking. It is of interest to note that the alterations in the analytical values tend to be maintained throughout the period of 8 weeks when the cows were put out to pasture and the giving of oils had ceased; reference has already been made to the sharp rise which occurred in the yield of fat.

It is difficult to draw conclusions as to the passage of oils into the butter from such figures as are provided by the analytical values. It will be seen that quite marked variations occurred, even in the first period of the experiment, when the cows were receiving the fixed basal diet only. Hence there is no justification for taking the value of any given constant at the beginning of a period of oil feeding as that which would have been maintained had no oil feeding occurred, for the variation, even when the animals were on a fixed diet, demonstrates that there are factors which cannot be controlled. Figures representing the difference' between the constants for any analytical value at the beginning and end of an oil period are valueless, unless they show a considerable percentage difference from those at the commencement. With the stipulation that these differences give only an approximate idea of the change in composition induced by the oil feeding, we may consider them for the cod-liver oil period only; they are 'not sufficiently definite during the arachis and coconut oil period to be of value. The following table gives the average values of the constants from the butter-fats of all three cows at the beginning and end of the cod-liver oil period.

Reichert Wollny value	Saponification value.	Iodine value.	Refractive index.
29·6–24·9	237·1–221·3	26·9–51·4	1·4535–1·4556

If we assume that the modification of the butter fat indicated by these figures was due to passage of the complete oil into the milk, and that there was no preferential passage of any individual constituent, we may calculate what quantity of cod-liver oil added to butter fat, of the average composition possessed by those at the commencement of period 4, would yield a butter fat having the analytical values given above at the end.   This gives 12 per cent. in the case of the iodine value, 30 per cent. for the saponification value, 7 per cent. for the Reichert-Wollny value, and 11 per cent. for the refractive index.   Three of these figures agree well, but it is to be emphasised that no conclusion can be drawn from these calculations as to whether individual constituents of the oil have been preferentially passed into the fat, although they serve to bring out the change caused by the cod-liver oil feeding.

A further point of interest is that the amount of fat in the milk produced by the cows per day during the time when they were receiving the basal ration only, without oil supplement, was from two to two and half times the amount actually present in the diet, yet when the amount of cod-liver oil given per day was about 75 per cent. of the amount of fat yielded per day, only about 12 per cent. of this oil appeared in the milk.

As remarked above, it was hoped by careful fractionation of the methyl or ethyl esters of the fatty acids of the butters to obtain more definite information regarding the passage of fatty acids from the food into the milk, particularly in the case of acids which are present in arachis and cod-liver oil but normally absent from butter fat.

FRACTIONATION OF METHYL ESTERS.—The method of fractional distillation of the esters from the fatty acids has been employed in the case of butter fat by numerous investigators, including Smedley (1911–1912), Crowther and Hynd (1917), Holland and Buckley (1918), Holland and others (1923), and with other oils by Bull (1906), Elsdon (1913).   The methyl esters were prepared by passing dry hydrogen chloride gas into a solution of the dry fatty acids obtained in the usual manner in methyl alcohol boiling under a reflux condenser; the methyl alcohol had been allowed to stand over fresh quicklime for several days and had been redistilled from lime twice.   The esters were removed by means of a separating funnel, more methyl alcohol added, and further separation made after again heating under a reflux condenser with hydrogen chloride.   The united esters were then dissolved in ether and washed with successive small quantities of distilled water, to remove the dissolved hydrochloric acid, until the washings did not affect blue litmus.   The ether solution was then allowed to stand overnight in contact with anhydrous sodium sulphate, and the clear yellow solution filtered from the sodium sulphate, which was then washed with ether distilled from sodium.   The combined filtrate and washings were freed from the bulk of the ether by heating on a water bath to 100° C., the flask and its contents were then heated to 130° C. in order to remove the last traces of ether.   This method of preparation resulted, of course, in the loss of the greater part of the lower esters.   No effort was made to avoid this in this preliminary work, because it was felt that the real difficulty

would be encountered, not in the separation of the esters of the lower acids, but in obtaining those of the acids with more than 6 carbon atoms in a pure state by fractionation, and if the quantitative separation of the lower acids later proved impossible, much information could be obtained by carrying out the Reichert-Meissl, Polenske and Kirschner determinations.

The esters were then submitted to fractional distillation in a double-necked flask, one limb of which contained a five-pear fractionating column. The distillation was carried out at a pressure of 15 mm. or thereabouts, such as is usually given by water-pumps, and every effort was made to keep the pressure as constant as possible. The whole of the upper part of the flask was well lagged with a thick layer of tightly packed asbestos, and the distillate was received into a vacuum receiver of the Bruhl type, containing twelve tubes, each of 15 c.c. capacity. It was thus possible to receive at a maximum 180 c.c. of esters without any intermission in the heating or increase in pressure. An approximate idea of the progress of the fractionation was obtained by determination of the iodine value of the individual fractions. At the end of the first fractionation the contents of the tubes which contained esters boiling at points on either side of the boiling point of that of each pure ester were mixed, and a second fractionation then carried out. At the end of this fractionation a similar procedure was adopted, and this was repeated four times in all.

Typical of the fractionations may be quoted the following, which gives the results obtained during the first distillation of 150 grms. of methyl esters:

Fraction.	Pressure.	Temperature °C.	Iodine value.
1	17 mm.	70–100	0
2		101–130	10·2
3		131–160	20·3
4		161–163	22·7
5		164–175	23·8
6		176–190	32·9
7	15 mm.	191–196	35·1
8		197–200	40
9		201–204	40·1
10		205–209	43
11		210–213	46·5
12		214–218	50·8
13		undistilled residue	61·7

The figures obtained for the iodine value after three more distillations of this material were not substantially different from those quoted in the foregoing list.

Various modifications were made in the flask by alteration of the type and length of the fractionating column, but without any real improvement in the purity of the fractions, as judged by their iodine values. It was considered, therefore, that it would be impossible to obtain by fractionation pure esters which could be weighed as individual substances, and, after much further work, we were reluctantly forced to adopt the opinion that, as a method of quantitative separation of the fatty acids of butter fat, the process is of little practical use. The method

employed by Holland and others (1923) is substantially that published by Holland and Buckley in 1918, and very similar to that described by Crowther and Hynd (1917). The former report the results of a series of experiments on the composition of butter fat from cows at various stages of lactation, and on different diets. They distil the ethyl esters of the fatty acids of butter fat at atmospheric pressure once only; they receive into flasks six fractions, and, from the weights of the contents and their saponification and iodine values, they claim to be able to give an analysis of butter fat to three decimal places per cent. Their calculations are based on the assumption that each fraction will contain oleic ester and the esters of two succeeding members of the saturated series of esters and no others. Thus the first fraction will contain butyric, caproic and oleic esters, the second caproic, caprylic and oleic esters, and so on. Each saturated ester will thus appear partly in each of two successive fractions and no others. If this be admitted, and also the assumption that oleic ester is the only unsaturated ester present, then a determination of iodine value will provide a means of calculating the amount of oleic ester in each fraction. This weight, on subtraction from the weight of the fraction, leaves the combined weight of the two saturated esters, and, from a determination of the saponification value, the weight of each of these can be found algebraically. From our own experience we think that objection should be taken to some points of this work. Thus the iodine and saponification values are given to three decimal places, which would imply an accuracy in these determinations which we do not believe it possible to attain. Further, the temperature which is reached in the distillation of ethyl esters at atmospheric pressure (the last fraction is received at a temperature of 365° C., which would probably mean one of over 400° C. in the flask) is such that considerable decomposition is bound to occur, for we found that in a similar distillation of the esters from palm-kernel oil, considerable loss occurred on account of decomposition, and even in distillation at 15 mm. pressure, the loss on this account is not negligible. Nor does it appear wise to weigh each fraction to four places of decimals, in view of the fact that it is collected merely by means of a flask exposed to air and standing at the end of a condenser. On these grounds alone it would appear that the authors have ascribed to their work a degree of accuracy which cannot be reached in work of this type, and hence there is no justification for their expression of their results of the analysis of the fatty acids of butter fat to three decimal places per cent. when such things as decomposition, presence of unsaponifiable matter, etc., have been neglected.

The question whether each fraction will contain two saturated esters and oleic ester only is of much greater importance, and the whole method employed by these workers is dependent on this being true. Crowther and Hynd (1917) have reported that this is borne out by experiment. In a study of the question, they made an artificial mixture of the methyl esters corresponding to an approximate composition which such esters prepared from true butter fat would have, distilled this mixture up to 150° C. at atmospheric pressure, taking adequate precautions to ensure no loss of the lowest esters on account of their volatility, and then the remainder in vacuum. This fractionation was repeated three times, and, working

on the principle that each fraction contained two successive saturated esters and oleic ester and no others, the analysis found by this method corresponded extremely closely to the actual composition of the mixture of esters as made up. Their recovery was so high as 95 per cent. Thus the method would appear valid, but it seems to us fundamentally unsound to assume that the vapour pressure curves of the saturated esters should differ so greatly from that of oleic ester that each of the former substances would appear in two successive fractions and no others. Surely, if it is reasonable to expect that oleic ester appears in the lower fractions, palmitic and stearic esters, etc., will tend to behave in somewhat similar manner, but the inability to detect them in so ready a manner as is given by the iodine value in the case of oleic ester is due to their lack of specific chemical properties. Further, even if the method be accepted, it appears very difficult to decide at what point each stage of the fractionation should come. Crowther and Hynd say that they found it necessary to fractionate three times, whilst Holland and Buckley's statement that "the object was to secure fractions that did not contain more than two esters in addition to oleic ester, and, furthermore, adjacent fractions should contain approximately half the saturated ester appearing in each," and the fact that they fractionate once only, would seem to introduce an element of chance which renders the method of no value for quantitative work.

It is to be pointed out that, because the saponification value of any fraction of the working, after allowance for oleic ester, lies between those of two adjacent esters, there is little, if any, justification for saying that these two esters alone are present, as it would be possible to make many mixtures of esters having any given saponification value and containing varying quantities of several esters.

The conclusion to be drawn from our experience with fractionation of the esters of the butter fat fatty acids is that, as an exact quantitative method, it is of little value. Furthermore, we are inclined to question whether the many published figures for the composition of butter fat often given to two or three places of decimals per cent., which are based on the ester method, are of more value than mere approximations. This method would appear to be of the same order of accuracy as E. Fischer's ester method for the analysis of proteins; that is, it can yield very valuable information if its limitations are recognised.

REFERENCES.

Holland, Garvey, Pierce, Mercer, Archibald, and Durbar. *J. Agric. Proc.*, Washington, **1918, 12,** 719.
Holland and Buckley. *J. Agric. Proc.*, Washington, 1923, 24, 365.
Crowther and Hynd. *Biochem. J.*, 1917, 11, 139.
Drummond, Coward, Golding, Mackintosh, Zilva. *J. Agric. Sci.*, 1923, 13, 144.
Drummond, Channon, Coward, Golding, Mackintosh, Zilva. *J. Agric. Sci.*, 1924.
Hopkins. *Biochem. J.*, 1920, 14, 721.
Smedley. *Biochem. J.*, 1911–12, 6, 451.
Bull. *Ber.*, 1906, 39, 3570.
Elsdon. ANALYST, 1913, 38, 442, 8; *ibid.*, 1914, 39, 455, 78.

DISCUSSION.

Mr. E. M. HAWKINS said he thought it was a pity some normal cow had not been put alongside the test cows, for it was well-known that the Reichert value

of the butter from cows' milk towards the end of the lactation period sometimes ran very low; he suggested that the drop at the end of the curve might be due to this.

Mr. E. R. BOLTON, having complimented the authors upon the utility of their work, raised the question as to how far the vitamin strength *per se* of an oil affected the fatty composition of the butter, and as to whether the same given oil (rich in vitamin, or free from vitamin) would have a similar effect.

Capt. J. GOLDING, commenting on the paper, emphasised the practical importance of these discoveries to dairy farmers. The winter feeding of dairy cows required revision in the light of these newly discovered facts. The advantages of meadow hay for winter feeding as a better source of vitamin *A* than seeds hay was a point of the greatest practical importance, and might well be a factor in maintaining the food value of our winter milk. Many American workers had found a negative calcium balance when using sun-dried alfalfa hay.

Mr. G. R. THOMPSON enquired whether any attention had been given to any relation between the specific gravity and the melting point of the fats.

Dr. J. C. DRUMMOND, in reply, said that they had brought the paper before the Society very largely because they had felt that their six months' effort had been wasted. According to text books it was a relatively simple matter to get a satisfactory fractionation of methyl esters.

The chart which had been thrown on the screen giving the figures obtained by the American workers had caused amusement, but those workers were on the staff of an important Experimental Station. Not only were their figures published in a responsible journal, but they were reproduced in the text-books, and he felt it was time these inaccuracies were exposed. Regarding the point raised by Mr. Bolton: this had not actually been studied. As far as they knew, vitamin *A* had no direct influence on the composition of the milk-fat. In reply to Mr. G. R. Thomson's question, they had not observed any correlation between the specific gravity and the melting point of the butter. Butters of cows fed with cod-liver oil were tested for palatability, and, although the butter tended to be rather insipid, it had no objectionable flavour.

The Composition and Decomposition of Eggs

BY R. T. THOMSON AND JAMES SORLEY.

(*Read at the Meeting, March 5, 1924.*)

COMPOSITION OF EGGS.—During recent years we have made a large number of analyses of eggs, and in our experience there is reason for revision of some results which have been published. This we propose to do in the present paper, as well as to record some further data.

The method we adopt for the estimation of fat or oil in whole egg (edible portion) or yolk is as follows:

A weighed quantity of the sample is dried at 100° C. and the residue macerated with a flat-headed rod in a basin, using methylated ether as the solvent. The ethereal solution is decanted through a small filter, and the non-fatty solids treated repeatedly with small quantities of ether until the fat is completely extracted, the fat being recovered in the usual way. Jean states that different solvents give varying percentages. His figures for dried yolk are as follows:

					Fat extracted Per Cent.
By petroleum spirit	..	..	..	..	48·24
By ether ..	..	..	..	..	50·83
By chloroform	..	..	..	..	57·66

We, on the other hand, find that extraction is complete, whichever of the three solvents is used.

When petroleum spirit is used as the solvent it is advisable to have the sample absolutely dry, but with ether the drying need not be perfect. In the case of chloroform the addition of sand or other inert substance is necessary, as otherwise the non-fatty matter floats in the solvent, thus rendering extraction very difficult.

We may note here that in extracting yolks or whole eggs which have been preserved with 1 to 2 per cent. of boric acid a little of that acid, amounting usually to about 0·15 per cent., is extracted, and is obtained in the fat. It can, however, be expelled by adding methyl alcohol to the fat, when the boric acid is vaporised as methyl borate at 100° C. Chloroform only extracts traces of boric acid.

Further tests were also made with the object of extracting the fat with the solvents in a separator, the yolk being previously mixed with about ten times its weight of water. The extraction with petroleum spirit or ether was troublesome, and with chloroform practically impossible, owing to emulsification taking place. It was found that several treatments with ether extracted all the fat, but that petroleum spirit only yielded a very small percentage. Samples of hen's yolk and duck's yolk gave the following results:

Fat extracted	Hen's Yolk. Per Cent.	Duck's Yolk. Per Cent.
By ether	31·10	36·20
By petroleum spirit	0·48	0·43

Chloroform applied to the undiluted yolk extracts the fat fairly well.

We have made various analyses of hens' and ducks' eggs, but the results for fat, proteins, etc., come within the limits recorded in various works. Our aim was chiefly concerned with the change which takes place in the fat of yolks when kept for a period, and the percentage of free fatty acid in a perfectly fresh egg was of importance A hen's egg, the analysis of which was commenced one hour after laying, was tested, with the following results:

	Yolk. Per Cent.	White. Per Cent.
Fat	34·63	trace
Other organic matters	16·97	11·04
Mineral matter (ash)	1·49	0·83
Water	46·91	88·13
	100·00	100·00
Free fatty acids (as oleic acid) ..	0·59	—
Free fatty acids in the fat ..	1·72	—
Acid value	3·42	—
Unsaponifiable matter in the fat ..	4·28	—

This yolk was exceptionally rich in fat, but other samples did not show above 32 per cent. The yolk in this egg formed 38·4 per cent. of the whole edible portion, but in other eggs the amount of yolk ranged between that figure and 43·3 per cent.

The free fatty acids are low, as was to be expected, but the unsaponifiable matter is much higher than any recorded results. For this item a figure as low as 0·2 per cent. is stated by Vignon and Meunieur, whilst other observers give 1·5 and 3·0 per cent. of cholesterol, which forms part of the unsaponifiable matter. For the fat of ducks' eggs, Vignon and Meunieur give 2·7 per cent. of unsaponifiable matter.

The estimation of unsaponifiable matter was made by saponifying in the usual way and extracting with methylated ether, which is our usual procedure. In order to determine whether different solvents might give varying results, we extracted the fat from a hen's egg and a duck's egg, and carried out extractions with methylated and petroleum spirit respectively. The results, as well as those for other constants, are recorded below:

	Hen's Egg. Per Cent.	Duck's Egg. Per Cent.
Unsaponifiable matter (petroleum spirit)	0·36	0·28
Unsaponifiable matter (methylated ether)	3·75	4·27
Iodine value (Wijs)	74·73	75·51
Saponification value	183·79	180·90
Acid value	4·47	5·70
Free fatty (oleic) acid, per cent. ..	2·25	2·87
Reichert-Meissl value	0·62	0·27
Polenske value	0·28	0·25

It has been generally assumed that petroleum spirit extracts all the unsaponifiable matter from fats or oils, but this is very far from being the case with egg fat, and it is quite possible that other fats may show the same result. The case mentioned above, where only 0·2 per cent. of unsaponifiable matter was obtained, is probably due to petroleum spirit having been used for the extraction. The iodine value for hen's egg fat is consistent with some recorded figures, but 37·4 has been given for duck's egg fat. The other constants practically agree with the recorded results, which are extremely few. Other results for hen's egg fat were 4·54 and 4·28 per cent., and for duck's egg fat, 4·86 and 4·05 per cent. of unsaponifiable matter.

We have also directed our attention to the constants for fat in Chinese preserved hen's egg yolk, and our results are stated below:

	No. 1. Per Cent.	No. 2. Per Cent.
Unsaponifiable matter (ether) ..	3·64	4·57
Iodine value (Wijs)	78·51	84·20
Saponification value	186·10	184·30
Acid value	10·42	51·88
Free fatty (oleic) acid, per cent. ..	5·24	26·08
Reichert-Meissl value	2·5	0·9
Polenske value	0·4	0·7

ample No. 1 was in good condition, but No. 2 was about 2 years old, and had
posed, assuming a thick or pasty condition. Another sample yielded
4·73 per cent. of unsaponifiable matter.

A point of some interest that we have not seen referred to specially is the
reaction of yolk and white of egg to indicators. A fresh hen's egg, and one 7
months old, gave the following results:

White.		Hen's Egg (Fresh). Per Cent.	Hen's Egg (7 months' old). Per Cent.
Alkalinity (as NaOH) by methyl orange	..	0·08	0·51
Alkalinity (as NaOH) by phenolphthalein	..	0·02	0·08
Yolk.			
Acidity (as SO₃) by phenolphthalein	.. ..	0·15	—

It is impossible to say what the alkalinity of the white, or the acidity of the
yolk, is due to, as the effect of proteins and other constituents on the indicators is
not known. The yolks appeared to be somewhat acid to methyl orange, but a good
end-point was not obtainable. It may be noted that when yolk is treated with a
10 per cent. solution of sodium chloride, the fat extracted with ether, and the
vitellin precipitated by addition of water, the filtrate gives practically the same
acidity as the original yolk. The above results were for an egg that was certainly
newly laid, but higher alkalinity is obtained in the white according to the age of
the egg. The acidity of the yolk, except that due to the free fatty acids, does not
increase. Ducks' eggs give practically the same results as hens' eggs for alka-
linity of the white and acidity of the yolk. The ash of hens' eggs was also tested,
with the following results:

White.			In Ash. Per Cent.
Alkalinity (as NaOH) by methyl orange	..	..	6·31
Alkalinity (as NaOH) by phenolphthalein ..		..	2·24
Yolks.			
Acidity (as SO₃) by methyl orange ..	..	..	2·65
Acidity (as SO₃) by phenolphthalein	..	..	25·50

The acidity of the yolk ash towards methyl orange indicates that there is free
phosphoric acid in it, to the extent of 4·7 per cent. of phosphoric anhydride.
Similar results were obtained with ducks' eggs.

DECOMPOSITION OF EGGS.—When a shell egg is kept for a considerable time,
decomposition sets in. The white decomposes and the yolk also undergoes de-
composition. The former decomposes much more quickly than the latter, but in
this paper we have only concerned ourselves with the decomposition of the yolk.

It is not generally known that when the yolk is kept for some time, fermen-
tation sets in and free fatty acids are produced from the fat. The yolk of an egg,
tested 1 hour after laying, showed the presence of 1·72 per cent. of free fatty acids
(stated as oleic acid) in the fat, whilst after three months the amount of free fatty
acids is very little increased. After one year, this figure rises to 3·12 per cent., and
at the end of two years to 5·15 per cent. These figures are the average of a number

of tests, as we found that the free fatty acids in two eggs laid at the same time varied slightly.

The reason for this decomposition proceeding so slowly is that the yolk is well protected inside the white of egg.

We also made experiments with fresh eggs which were shelled and placed in a bottle with a tight-fitting stopper. After one year the fat was extracted, and the free fatty acids were found to be 85·5 per cent., calculated on the fat.

Further, a hen's egg of uncertain age, but which was unmistakably bad, was found to contain 17·3 per cent. of free fatty acids. The shell of this egg was cracked, and to this cause we attribute this high result.

Experiments were also made with Chinese hens' egg yolks, which are shipped to this country in bulk, without shells.

Two samples were tested with the object of ascertaining how quickly the free fatty acids are produced. The tests gave the following results:

	Free Fatty Acids.	
	Prime Quality. Per Cent.	2nd Grade Quality. Per Cent.
1st test	6·5	12·2
2nd test, after 6 weeks	7·4	13·2
3rd test, after 6 months	11·2	15·9

The above results show that free fatty acids are produced with time, but we were quite unable to find any definite relationship between time and production of free fatty acids. Our tests showed that a preservative such as boric acid slowed down the production of free fatty acids very considerably, but not altogether, as the samples of Chinese yolks tested above, were preserved with boric acid. On one point we were able to gain definite information, namely, that free fatty acids are produced more quickly in the summer than in the winter.

A series of tests with imported yolks showed that when the free fatty acid figure became high, the eggs were practically useless for whisking.

DISCUSSION.

Mr. M. S. SALAMON commented on the large amount of data—convenient for reference—which had been collected in the paper. The authors stated that it was immaterial which solvent was used for the extraction of the fatty matter, but, in his opinion, with petroleum spirit the extraction was by no means complete. The extractions were contrary to the methods employed by earlier workers, but confirmed the known fact that a larger extract was obtained with chloroform than with any other solvent.

The characteristics of chloroform-extracted yolk differed entirely from those of yolk extracted with any other solvent. He thought it a pity the authors had not returned the percentage of nitrogen in the fat, which might possibly have explained the divergence in the results with petroleum spirit and ether. This difference was not found with olive and similar oils, which differed very materially from an oil or fat, such as egg oil, that contained a large percentage of lecithin or basic substances.

Regarding the authors' experiments on fatty acids, the speaker did not think there was any relationship between time and the percentage of fatty acid content; the authors did not appear to have differentiated between the fatty acids produced by decomposition, lipase, moulds or bacteria. Eggs contained different fatty acids which could be detected by differences in the flavour.

Mr. E. HINKS said that he doubted the advisibility of drying the egg alone and extracting the dried residue; if the egg were dried as said, both the drying and subsequent extraction were facilitated. With regard to the unsaponifiable matter, petroleum spirit gave erroneous results with any oil containing much cholesterol, a fact which no doubt accounted for the low figures given by the authors. He agreed with Mr. Salamon's view that, although the authors advise methylated ether for extraction of the oil, their figures shewed that chloroform was the best solvent.

Dr. H. E. COX said he considered the method of drying the original egg as important as the method of extracting it. He had recently read a paper in an American journal in which the extract had been dried in a water oven for an hour. This, in his opinion, was a most undesirable method, the better way being to dry in vacuum over sulphuric acid. The authors' figures were interesting, but would have been much more so had they stated the amount of nitrogen and phosphoric anhydride they had found. When one extracted with chloroform (as required by the Produce Brokers Contracts) a definite statement should be made as to the method of drying. If the chloroform extract were dried in the oven, there was a long-continued loss of weight.

Mr. G. RUDD THOMPSON said that the proportion of extracted fat with the various solvents was very largely a question of the mode of drying; if an egg were dried in a water oven it would be smelt everywhere, so that something was obviously being lost.

Simple, Useful Forms of Hydrogen Electrode.

## BY F. J. CONSIDINE.

(Read at the Meeting, May 7, 1924.)

FOR rapid hydrogen ion determinations, especially in the case of biological fluids, the minimal surface contact principle, utilised in the electrodes of Walpole [1913] and of Barendrecht [1915] has much merit.

In large scale process control work, where approximate values suffice, the writer has used modified forms of the above electrodes, and these have proved so convenient and satisfactory that it seems worth while to describe them, as they may perhaps be found of special interest in connection with such inexpensive forms of potentiometer outfit as that well described by Monier-Williams (ANALYST, 1921, XLVI., 315).

Fig. 1 in the accompanying diagram shows a form of electrode for general use. The vessel, made from a broken test tube, is attached to the two-holed rubber stopper through which passes a Walpole platinum wire electrode with mercury drop connection, and also the lower end of the once-bent, stout-walled, capillary

bore glass tube. The horizontal portion of this tube acts as a fixed plunger within the sliding glass tube with well lubricated rubber sleeve, to form a simple suction filling device on the Barendrecht principle. The assembled apparatus is supported by a movable clamp grasping the horizontal portion of the capillary tube near the bend. This clamp, by movement upon a retort stand, permits the apparatus to be raised or lowered at will.

To use the electrode, the outer tube of the glass filler is withdrawn to the extremity of the plunger, and, controlled by the Hoffman screw clip, a current of purified hydrogen is passed through the system to expel air and saturate the
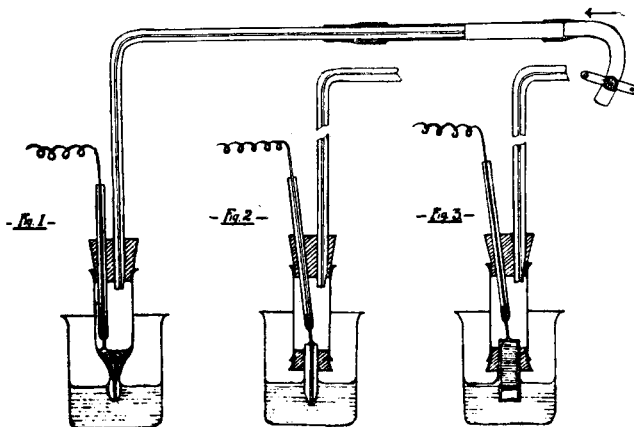
electrode. The fluid to be tested is then brought into contact with the lower end of the electrode vessel, the gas shut off, and, by appropriate manipulation of the filler, the fluid is drawn up into surface contact with the platinum point, when, after the usual adjustment of salt bridge and wire connections, under favourable conditions a voltage reading may be taken at once.

Fig. 2 illustrates a "micro" modification of the foregoing, and is used where very small quantities of a sample are to be tested. Quite satisfactory readings have been obtained with 0·1 c.c. or less of fluid with this electrode. The short, lower capillary tube is of neutral glass, and the stopper hole for the electrode is bored somewhat obliquely to facilitate adjustment of the platinum point directly over the upper orifice of the tube. Walpole's ingenious procedure for testing small quantities of fluid is used here. After thorough flushing of the apparatus with gas a drop or two of the sample fluid is cautiously drawn into the lower end of the short capillary tube, and saturated solution of purified potassium chloride from the vessel below is drawn up after the sample fluid until the latter just makes contact with the platinum point, when a reading can be taken in the usual way. This particular method of exposing the sample fluid to the gas seems to be very effective in securing a rapid equilibrium, as in some cases, where trouble was experienced in securing steady readings with other electrodes, this capillary system gave very rapid and reproducible results. Almost the only objection to this electrode for general use is the need for greater care in its manipulation.

Fig. 3 shows a simple modification of the capillary form and has been found useful for fluids of low conductance. Its use needs no special description.

The simple forms of electrode here described are very easily and cheaply made from materials always at hand, and without expert glass-blowing. They are very strong, the rubber stopper acting as a "shock absorber" in rough handling, and all parts are interchangeable and easily accessible for cleaning.

Some brief working notes in connection with these electrodes may perhaps be of interest.

Palladium chloride is used rather than the platinum salt for coating the platinum wire electrodes. The procedures of Clevenger [1919] for cleaning and coating such electrodes have been found excellent.

For testing the coated electrodes, the potassium hydrogen phthalate solution recommended by Clark [1920] has proved quite satisfactory and much easier to prepare than the buffer mixtures of Walpole, Sörensen, Palitzoch and McIllvaine.

A very simple and good salt bridge used is that described by McClendon [1918], consisting of a thin rubber tube filled with saturated solution of purified potassium chloride, and with ends plugged with pieces of a wooden match, previously boiled in a little of the same solution. This rubber salt bridge connects the sample fluid with a small container of saturated solution of potassium chloride, which also receives a glass siphon provided with a tap kept closed from a home-made Barendrecht [1915] pattern calomel half element. Such calomel half cells, prepared from carefully purified materials and with potassium chloride at full saturation, have proved remarkably stable and are very convenient in use. Protected from light by a covering of suitable paper, they appear to have a very long life. The cylinder hydrogen used is sufficiently purified by passage through Drechsel wash bottles containing alkaline permanganate, alkaline pyrogallol and distilled water. It has been found well to seal glass taps to the intake and outlet tubes of the Drechsel bottles. A "water balance" on the Kipp apparatus principle, in line between gas cylinder and scrubbing train, checks the evil of faulty cylinder valves. A single glass tap, mounted upon the "water balance," is connected by a T-piece with cylinder and scrubbing train, and, in case of a very intractable valve, it is well to disconnect the train while charging up from the cylinder.

REFERENCES.

Walpole. *Biochem. J.*, 1913, **7**, 410.
Barendrecht. *Ibid.*, 1915, 9, 66.
Monier-Williams. ANALYST, 1921, **46**, 315.
Clevenger. *Soil Science*, 1919, **8** [3], 217.
Clark. *The Determination of Hydrogen Ions*, Baltimore, 1920.
McClendon. *J. Biol. Chem.*, 1918, **33**, 19.

DISCUSSION.

Miss D. JORDAN LLOYD said that she much appreciated the simplicity of design of the apparatus shewn. The first hydrogen electrodes were made upon the simplest mechanical lines, and she regretted the present day tendency to design elaborate apparatus.

Mr. A. Chaston Chapman enquired whether it was necessary to pass the hydrogen for fifteen or twenty minutes in order to obtain a constant reading, as was the case with most of the standard electrodes. The apparatus appeared very much simpler than most of the forms in common use, and he thought that any simplification which could be effected without reducing the sensitiveness of the electrode was very desirable indeed.

Dr. H. E. Cox (who read the paper) said that he had not actually used the apparatus. The connection might be made and under ordinary conditions a voltage reading might be taken almost at once. It was important that the platinum point should only just touch the surface of the liquid and not be immersed to any depth therein.

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.

OLIVE OIL.

The writer has recently examined two samples of olive oil, each of which had been reported upon as adulterated. The results obtained may be of interest to other analysts.

Sample No. 1. This had an extremely bitter taste, not unlike that of strychnine, and left a burning sensation in the throat.

On analysis it gave the following figures:—Sp. gr., 0·9156; saponification value, 194·4; iodine value, 79·9; free fatty acids, 0·4 per cent.; and n_D^{40}, 1·4613; arachis and cotton-seed oils, absent; Baudouin's test (Furfural and HCl modification), a distinct red, changing to olive-green.

The bitterness and reaction in Baudouin's test seemed the only abnormalities. On referring to Thorpe's Dictionary of Applied Chemistry, Vol. IV., p. 701, I found the following:

"According to Bourquilot and Vintilesco, olives contain a glucoside, oleoeuropein—a yellow powder with bitter taste—hydrolysable by emulsin, which is present in the fruit, leaves and bark." And under Olive Oil, p. 702: "Olives intended for oil production are gathered just before maturity, as the oil obtained from the barely ripe fruit is much superior in quality to that obtained from fully-ripe or over-ripe fruits."

As it seemed possible that the two bodies might be present in this oil, I shook up some of the oil with water (A) and kept the mixture at a temperature of 80° F., together with some of the oil (B). At the end of ten days the separated oil from sample A was free from bitterness and acridity, and was bland and of a beautiful golden colour.

The analytical figures were very similar to those of the untreated oil, excepting that the free acidity was slightly reduced, and that Baudouin's reaction was almost negative (a very slight and fugitive pink).

Sample B was still bitter and acrid after 14 days in the incubator.

The writer suggests that the abnormal climatic conditions in Spain last year were not favourable to complete hydrolysis taking place before the fruit was pressed (hence the bitterness), and that emulsin and oleoeuropein being present in

some stage of hydrolysis, other than the bitter one, may account for the fact that some olive oils give a positive reaction in Baudouin's test.

Sample No. 2. This oil was reported upon as containing 30 to 40 per cent. of arachis oil, this conclusion, presumably, having been arrived at from its behaviour in the British Pharmacopoeia test. The writer has frequently experienced difficulty with this test during the winter months, when the temperature during the 24 hours sometimes falls many degrees below freezing point. As the result of some tests, he would suggest that the B.P. instruction should be altered to read "and kept at a temperature of 15·5° C. for 24 hours."

Some of the oil in question, afterwards reported upon as free from arachis oil (A), some mixed with 15 per cent. of nut oil (B), and some with 10 per cent. of nut oil (C), were subjected to the B.P. test, with this difference, that all three were kept in an incubator at a temperature of 15·5° C. for the required period. The following results were obtained:—

A. Entirely free from crystals; B. A heavy deposit of crystals; C. A very few crystals (so few as to leave one doubtful).

On putting flasks A and C into a water-bath and keeping them at a temperature of 12° C. for an hour, no crystals were deposited in A, but C (with 10 per cent. of nut oil) gave a heavy deposit.

F. F. SHELLEY.

OSMIUM TETROXIDE AS A REAGENT FOR THE GOLD-BEATERS' SKIN TEST FOR TANNINS.

C. AINSWORTH MITCHELL (ANALYST, 1924, 49, 162) has recently described a quantitative method for the colorimetric estimation of tannins which is based on the use of osmium tetroxide. I have, at the suggestion of Dr. Nierenstein, attempted to use this reagent in connection with the Gold-Beaters' Skin Test for Tannins, as worked out in this laboratory (Atkinson and Hazleton, *Biochem. J.,* 1922, 16, 516; ANALYST, 1923, 48, 38; Price, ANALYST, 1924, 49, 25), with the result that I have found it to be less delicate than ferrous sulphate or ferrous chloride as recommended by me. Whereas ferrous sulphate is capable of detecting 0·00005 grm. of gallotannin in 1 c.c. of water, osmium tetroxide detects only 0·0001 grm.

PHYLLIS H. PRICE.

BIOCHEMICAL LABORATORY,
        UNIVERSITY OF BRISTOL.

ARSENIC IN PRINTING INK.

DURING the examination of a sample of proprietary bread it was discovered that the violet ink used for the printing on the wrapper contained appreciable quantities of arsenic. As a result of this discovery a large number of coloured wrappers and labels and a number of coloured papers have been examined to discover whether they contained arsenic. In all, 51 samples have been examined, and of these, nine contained an appreciable quantity of arsenic. These nine samples consisted of two blue papers, five purple papers and two green papers. The quantity of arsenic in one paper faced with a purple colour was about one-third of a grain per square foot.

It was not considered advisable to approach the packers of these goods in order to discover the source of the colours used in the printing. The writer,

therefore, made careful inquiries in regard to the composition of printers' inks, and finally, through the courtesy of a large local firm of lithographic printers, he was able to procure nine samples of ink largely used in printing. These consisted of three blues, one violet, one water-violet, one red, two greens and one yellow. All these inks were practically free from arsenic, with the exception of one violet ink, which contained about 10 per cent. of arsenic, calculated as arsenious oxide.

Apart from any danger that might arise from the licking of such brightly coloured paper by young children, it would not seem advisable to allow the possibility of persons obtaining poisonous doses of arsenic without any record that such arsenic had been in their possession.

The presence of the arsenic is due to the use of arsenical lakes in the colours from which the printing ink is made. By the use of these arsenical lakes it is alleged that brighter colours can be obtained, but there seems to be no real reason for their use, especially when one takes into account the possible danger that might arise. This danger is not very urgent, as lakes generally are used on account of their insolubility.

One violet colour used in confectionery was examined, but was found to be free from arsenic. This is merely what was to be expected, as the type of soluble colour used in food is quite different from the lakes used in colour printing.

G. D. ELSDON.

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 interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.

COUNTY OF LANCASTER.

ANNUAL REPORT OF THE COUNTY ANALYST FOR 1923.

OF the 4984 samples submitted during the year, 4640 were from the Police, 306 from local authorities, and 2 from other persons. Of the samples submitted by the Police, 365 (7·87 per cent.), and those from local authorities 28 (9·15 per cent.), were adulterated or not up to standard. The total percentage of serious cases, however, was only 2·77 per cent.

MILK.—The number of samples purchased was 3049, and of these, 338 (11·08 per cent.) were adulterated, deficient or dirty. Cow dung exceeding 5 parts per 100,000 was found in 0·62 per cent. of the samples, and in excess of 2 parts, but not of 5 parts, in 2·79 per cent.

In 29 cases milking of the cows was supervised. The highest percentage of fat then found was 5·02, and the average 3·42; whilst the highest and average amounts of solids-not-fat were 9·65 and 8·60 per cent. respectively.

INFORMAL SAMPLES.—The 4301 samples taken without notice included 2456 milks. Of these, 75 milks and 18 other samples were adulterated.

SAMPLES OTHER THAN MILK.—Five samples of rice contained from 0·1 to 0·4 per cent. of talc. One sample (of 90 examined) of baking powder contained 9 per cent. of calcium sulphate, and one sample of cream of tartar (of 61 examined) contained 28 parts of lead per million.

Ten samples of jam contained salicylic acid in amounts varying from 0·17 to 4 grains per lb. One sample of potted meat, labelled "Free from Preservatives," contained 1·5 grains of boric acid per lb. Official cautions were sent to the manufacturers of potted lobster devoid of lobster, of potted meat containing 30 per cent. extraneous moisture, and of lemon cheese and lemon curd containing 2·9 and 1·9 grains of salicylic acid respectively.

DRUGS.—Four samples of magnesia consisted of magnesium carbonate instead of the oxide. One sample of borax, sold by a grocer, contained 0·5 grain of arsenic per lb.

W. COLLINGWOOD WILLIAMS.

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.

NOTIFICATION OF WARRANTY.

ON May 15th a firm of druggists was summoned by the Southwark Borough Council at the Lambeth Police Court for selling quinine sulphate which was not of the nature, substance, and quality demanded.

It was explained to the magistrate (Mr. S. Fleming) that the Council's inspector had asked for quinine sulphate and had been supplied with quinine hydrochloride. The defendants pleaded a warranty, but the notice had been sent to the Town Clerk, and not to the purchaser as required by the Sale of Food and Drugs Act, 1889.

For the defence it was urged that the wholesale firm which had supplied the drug had made a mistake, and had sent a more expensive article (quinine hydrochloride) instead of the quinine sulphate ordered. With regard to the legal point, it was submitted that what had been done was a sufficient compliance with the statute.

The Magistrate held that there had not been compliance with the statute, and ordered the defendant to pay 12s. 6d. costs.

There was a second summons against the defendants for selling bicarbonate of soda as purified borax. For the defence it was stated that a mistake had been made. A fine of £5 with £2 16s. 6d. costs was imposed.

RAG-FLOCK ACT CASE.

ON April 29th a firm of upholsterers was summoned by the High Wycombe Borough Council for unlawfully having in their possession flock manufactured from rags intended to be used for upholstery, which flock did not conform to the standard of cleanliness prescribed by the Rag-Flock Regulations, 1912, made by the Local Government Board.

The High Wycombe Town Clerk, for the prosecution, explained that the sample in the present case contained 904 parts of chlorine per 100,000, whereas, under the Rag-Flock Act, only 30 parts per 100,000 was permissible.

Evidence in support of the prosecution was given by Mr. A. P. Davson, F.I.C., to the effect that this was the dirtiest sample of rag-flock he had ever analysed. It consisted of coconut fibre and bagging. He had not discovered any animal fibre, nor had he tested it for grease, but there were particles of foreign dirt. From its appearance he concluded that the material was principally made from gunny bags, with a much smaller amount of coconut fibre. In his opinion, the high proportion of chlorine indicated that the sample was dirty.

A flock manufacturer gave evidence that the sample was composed of coconut fibre and bagging unwashed. Ordinary rag-flock, when washed, lost about 35 per cent. in weight, and there was also the cost of drying. Hence, in his opinion, there was a difference of £10 per ton in the cost of producing unwashed and washed flock.

Another flock manufacturer stated that the sample consisted of about 75 per cent. of bagging and 25 per cent. of fibre. In his opinion the material was unwashed and was unsuitable for upholstery.

The defendants stated that they were under contract for a supply of ordinary rag-flock, but that, owing to a breakdown at the mills, the flock-manufacturers were unable to supply them with their usual material, but said that they could supply a good fibre mixture. The manager of the mills had stated that his firm were prepared to take all risks, and that no guarantee was required, since the fibre mixture did not in any way come under the Rag-Flock Act. Witness produced his invoice showing the small quantities of the fibre mixture which had been purchased, also a letter from the manufacturers confirming the guarantee.

The manager of the mills confirmed the statements of the preceding witness. The material, he said, did not require a guarantee, since it did not come under the Act. He had about 40 customers for this material in Wycombe, but had stopped selling it pending the result of the case.

A consulting chemist called for the defence stated that he had found the sample submitted to him to be a clean fibre mixture, which appeared to consist of coconut, hemp, and jute. He had not detected any woven material, and the sample was also free from wool and cotton. In his opinion the sample did not come under the provisions of the Rag-Flock Act. The high chlorine content was to be expected in view of the fact that coconut fibre was prepared by steeping in salt water. The amount of soluble chlorine was a rough indication of the state of cleanliness of a flock, but had no meaning in the case of a fibre mixture containing coconut fibre.

Counsel for the defence said that if any offence had been committed it was not wilful, and that there had been no intention of evading the Act. He quoted various cases, including one in which Mr. Justice Avory remarked that flock must be manufactured from rag in the ordinary sense of the word.

The Town Clerk, in reply, pointed out that in the case of *Cooper* v. *Swift* it had been laid down that rag-flock was made of woven material, and that even the defence had admitted that there were pieces of woven material in the sample.

The magistrates held that the charge had been proved, and imposed a fine of £7 with £3 costs. Notice of appeal was given.

Meteorological Office, Air Ministry.

ADVISORY COMMITTEE ON ATMOSPHERIC POLLUTION.

REPORT ON OBSERVATIONS IN THE YEAR ENDING MARCH 31ST, 1923.*

THE STANDARD GAUGE.—Thirty-five of these gauges, in which atmospheric impurities accumulate with the rain, were in operation during the year under 16 authorities. The results are expressed, as in the last report, in terms of metric tons per 100 sq. kilometres, or in grms. per 100 sq. metres. Generally speaking, a marked deduction in atmospheric pollution was noticed during the year, and a table is given showing the places with the highest and lowest deposits, including tar, for summer (S) and winter (W), 1922–23:—(S) Newcastle, 242 m.t.; Birmingham, 20 m.t.; (W) Newcastle, 195; Glasgow, 20. *Insoluble ash*: (S) Liverpool, 5400; Rothamsted, 478; (W) Liverpool, 5890; Rothamsted, 231. *Carbonaceous matter*: (S) Blackburn, 2696; Southport, 427; (W) Liverpool, 3933; Rothamsted, 121. *Volatile salts*: (S) Glasgow, 2609; Birmingham, 398; (W) Glasgow, 1519; Birmingham, 262. *Soluble ash*: (S) Glasgow, 3280; Rothamsted, 421; (W) Hull, 2903; Rothamsted, 408. *Total solids*: (S) Rochdale, 16698; Rothamsted, 2298; (W) Liverpool, 13565; Rothamsted, 1140.

THE RECORDS OF THE AUTOMATIC FILTER.—This instrument, which measures, hour by hour, the amount of suspended matter in the air, is illustrated by diagrams and numerous graphs showing the suspended impurities on ordinary week-days, Saturdays and Sundays for various parts of London and Glasgow. An increased number of these filters has been in operation during the year with satisfactory results.

THE JET DUST COUNTER.—This counter, described in the previous year's Report (ANALYST, 1924, 49, 35) has been further tested for efficiency. A double-slot arrangement was found satisfactory when the deposit was too thick for examination in foggy weather with the single slot, and details for mounting and counting the records and illustrations of a portable instrument are given. A table of results obtained in Westminster, Cheam, Bloomsbury, S. Kensington, Kew Gardens, Grimsby, Brighton, Hull, etc., is given, and some of the typical results are discussed in detail. Some very interesting facts are brought to light, such as, for example, the sudden appearance of large numbers of mould-cells in the air in autumn; the occasional presence of crystals of soluble salts in the air, and the occurrence of spherical particles of a glass-like nature, the origin of which is at present uncertain. There is no doubt that the extended use of this instrument is being, and will be, of very great service in elucidating problems of air impurity. For instance, records already suggest that the haze often met with over the South of England may be produced in the manufacturing cities of the Midlands and North; and it is hoped to investigate, with the help of the jet dust counter, such problems as the relation of impurity to strength of wind, of bacterial contents to smoke, and so on.

EFFECT OF ATMOSPHERIC POLLUTION UPON VISIBILITY.—Three methods of observation have been brought to bear upon the problem, viz. the method of the illuminated hollow cube; of the searchlight with contrast photometer; and a combination of the two. Details of results are given, and satisfactory agreement was obtained by the different methods. It is expected that valuable information will accrue from observations on variations of obstruction of light by winter smoke fogs.

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

New Methylated-Spirit Regulations.

THE Commissioners of Customs and Excise have issued the following notice to makers of methylated spirits regarding the change in the composition of methylated spirits which came in force on May 1:—

1. The Commissioners of Customs and Excise hereby give notice that all mineralised methylated spirits made on and after May 1 must consist of: Plain British spirits or unsweetened foreign spirits or rum (90 per cent.), wood naphtha or methyl alcohol (9½ per cent.), and crude pyridine (½ per cent.), and must contain, in addition to this mixture, three-eighths of one per cent. by volume of approved mineral naphtha or petroleum oil, and not less than 0·025 of an ounce of aniline dye (methyl violet) for each 100 gallons of spirits.

2. Crude pyridine which conforms to the following specification will be regarded as suitable for making mineralised methylated spirits:—

(1) It should consist of the bases derived from coal tar, and should not be more deeply coloured than a solution of two c.c. of decinormal iodine dissolved in one litre of water.

(2) It should mix readily and completely with spirits and should give a clear or only slightly opalescent solution when mixed with twice its volume of water.

(3) Ten c.c. of a 1 per cent. solution in water should produce immediately a distinct crystalline precipitate on vigorous shaking after the addition of five c.c. of an aqueous solution of cadmium chloride containing five grammes of the anhydrous fused salt in 100 c.c., and an abundant separation of crystals within ten minutes.

(4) A white precipitate should be formed when 10 c.c. of a one per cent. solution in water are mixed with five c.c. of Nessler's reagent.

(5) One c.c. of crude pyridine dissolved in ten c.c. of distilled water should require not less than 9·5 c.c. of normal sulphuric acid for neutralisation, using Congo Red paper as indicator.

(6) One hundred c.c. slowly heated under the conditions laid down for benzol for motor fuel by the British Engineering Standards Association (B.S. Specification 2 D. 15) should give a distillate of at least 50 c.c. at a temperature of 140° C. and of 90 c.c. at 160° C.

3. Every maker of mineralised methylated spirits must provide in the naphtha warehouse on his methylating premises a suitable vessel of a minimum capacity of 10 gallons for storing the crude pyridine.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Viscosimetric Study of Wheat Starches. O. S. Rask and C. L. Alsberg. (*Cereal Chem.*, 1924, **1**, 7–26.) The physico-chemical differences in the gelatinised pastes of wheat starches have been shown by means of their viscosities; the relation between viscosity and concentration is expressed by the equation log viscosity $= m \times$ concentration $+ \log b$, where m and b are constants. High viscosity is observed to be associated with low loaf-volume and protein content and with a high temperature in the locality of growth, and conversely. Starches from winter wheats have higher viscosity than those of spring wheats. For the determinations, 100 to 125 grms. of the flour were made into dough with tap water, washed free from gluten, and the starch first treated with 1 per cent. salt solution to dissolve globulins, then separated and washed, first with water then with alcohol and ether, with the aid of a centrifuge. The product so obtained was dried to constant weight over

calcium chloride under reduced pressure and, when stored, contained 3 to 5 per cent. of moisture.   The viscosity of pastes varying from 3 to 6 per cent. concentration was measured at 90° C. by a Stormer viscometer, in which a falling weight rotates a cylinder immersed in the viscous liquid; the resistance is measured and calibrated in terms of centipoises (this is practically equivalent to viscosity compared with water $=1\cdot0$).   The raw starch suspension was warmed by a small burner, with constant movement of the cylinder in the viscometer cup, and the viscosity observed for every 10° C. rise of temperature.   A decrease in viscosity occurs between 93° and 95° C., indicating complete gelatinisation.   The temperature was then allowed to fall to 90° C. and a number of determinations were made.   At this temperature the viscosity in 5·5 per cent. concentration varies between 50 and 213 centipoises, according to the class of wheat.   H. E. C.

Nutritive Properties of Wild Rice.   C. Kennedy.   (*J. Agric. Res.*, 1924, **27**, 219–224.)   Comparison has been made by chemical and biological analysis between wild rice (*Zizania aquatica*) and cultivated rice.   The chemical analyses of 4 samples of wild rice are as under:—

No.	Moisture. Per Cent.	Ash. Per Cent.	Protein. Per Cent.	Ether extract. Per Cent.	Fibre. Per Cent.	Starch. Per Cent.	Soluble Carbohydrates as dextrose. Per Cent.
1	7·74	1·09	13·36	0·46	1·39	65·26	2·98
2	7·85	1·38	13·97	0·89	1·41	61·69	3·69
3	8·93	1·17	14·62	0·72	1·94	60·47	2·33
4	7·83	1·25	14·40	0·66	1·29	62·03	2·93

Although these results show a high proportion of proteins in wild rice, both these and the mineral salts present are of a variety unsuitable for dietetic purposes. There is just sufficient vitamin *A* to prevent xerophthalmia, but not enough to promote growth.   Wild rice is somewhat superior to the cultivated variety as regards its content of vitamin *B*, but still is not an adequate food.   H. E. C.

Preserved Spinach.   L. Gobert.   (*Ann. Falsificat.*, 1924, **17**, 158–160).— Microscopic examination of a sample of preserved spinach suspected of adulteration with foreign leaves revealed the epidermis and parenchyma of the spinach leaf, round pollen grains, the rounded anthers (from 0·2 to 0·5 mm. in diameter and having a mammillary surface), and vestiges of the staminal threads. Only the male flowers were found, the absence of female flowers being probably explained by the practice, among the seed-growers, of uprooting the male plants after fertilisation.   Such plants are doubtless bought at a cheap price, and preserved, and although not adulterated, are of inferior quality, especially if more or less fibrous parts of the petioles should remain in the product.   Proper plant should, however, remove such fibrous material.   T. H. P.

The Fining of Wine with Potassium Ferrocyanide.   H. Bosselmann (*Zeitsch. Unters. Nahr. Genussm.*, 1924, **47**, 209–214.)   The practice of fining and removing iron from wine by means of ferrocyanide, as recently described in an

official pamphlet in Germany, may lead to the formation of small quantities of hydrocyanic acid. For the detection or estimation of hydrocyanic acid in wine the usual silver nitrate test is not satisfactory, either when applied directly or after distillation with dilute acid. The following process is recommended:— About 300 c.c. of the wine are heated to boiling in a round-bottomed flask connected with a ball reflux condenser. Through the boiling liquid is passed a stream of carbon dioxide which is led from the top of the condenser through small wash bottles containing (a) 30 c.c. of 6 per cent. sodium bicarbonate solution, (b) 10 c.c. of 0·01 N mercuric chloride solution acidified with 1 c.c. of dilute hydrochloric acid, and through 2 test tubes, each containing 10 c.c. of 0·01 N silver nitrate solution. The bottles (a) and (b) are heated in hot water baths to 90° C. and 50–60° C. respectively. When all the hydrocyanic acid is carried over, as indicated by testing with a further quantity of silver nitrate, the solution is back-titrated with ammonium thiocyanate, as in Volhard's method. The silver cyanide precipitate may be identified by the formation of Prussian blue in the usual way. Details are given of the experimental fining of certain wines. Free hydrocyanic acid is only found when an excessive quantity of ferrocyanide is added, and is apparently formed by the action of the organic acids present in the wine. When testing for hydrocyanic acid it is important first to remove any precipitate of Prussian blue which may be present; otherwise it will decompose at the boiling point.

H. E. C.

Fatty Acids of Camel Hump Fat. N. McClelland. (*J. Soc. Chem. Ind.*, 1924, **43**, 164 T.)—Values for the fat obtained from the hump of a fully-grown young camel (total 80 lbs.) were as follows:—Sp. gr. at 100/15° C., 0·872; m.pt., 35–36° C.; insoluble fatty acids, 92 per cent.; unsaponifiable matter, 0·4 per cent.; saponification value, 205–206; iodine value, 37·4; m.pt. of fatty acids, 45–46° C.; neutralisation value of solid fatty acids, 210–211. *Solid acids.*—The first main fraction (b.pt. 195–200° C.) was identified as ethyl palmitate by its melting pt., melting pt. of the methyl ester, and melting pt. and molecular weight of the free acid. The second main fraction (b.pt. 210–215° C.) was similarly identified as ethyl stearate. The residue (boiling above 215° C.) was found to consist of impure stearic acid. *Liquid acids.*—Acids yielding tetrabromides and hexabromides were not present. The liquid acid was converted, through the potassium and lead salts, into the acid, and this into the barium salt, which was recrystallised, and the acid liberated. The oily acid melted at 11° C., and had a molecular weight of 280, and was proved to be oleic acid. The approximate composition of the mixed fatty acids was found to be: Palmitic, 37; stearic, 16; and oleic, 47 per cent.

D. G. H.

Bellier's Reaction applied to the Detection of Adulteration of Cacao Butter. N. B. Clemencet. (*Ann. Falsificat.*, 1924, **17**, 146–153.)—When present to the extent of 10 per cent. in admixture with colourless, neutral vaseline oil, pure cacao butter should give a positive violet reaction with Bellier's reagent (1·5 grms. resorcinol in 1 litre of benzene), stable for about 10 minutes, but changing

gradually into pink. This reaction may, therefore, be used as a criterion of the purity of cacao butter, and, by determining the minimum quantity of a mixture capable of showing the reaction, as a means of estimating approximately the proportion of cacao butter present in such mixture. T. H. P.

The Kreis Test. G. E. Holm and G. R. Greenbank. (*Ind. Eng. Chem.*, 1924, **16**, 518.)—Linolic, linolenic, and ricinoleic acids give a reaction with the Kreis reagent when they have absorbed oxygen, but the proportion of colour produced is less than that obtained with oleic acid. As regards ricinoleic acid, the relative proportion of colour it will give is small, and the rate of absorption of oxygen is slow. The odours produced in the different oxidations show that oleic acid is the main constituent of fats and oils that is concerned in the production of tallow flavour or rancidity (*cf.* ANALYST, 1923, **48**, 608). W. P. S.

Nature of the Oil of Soya Bean Miso. R. Kodama. (*Ind. Eng. Chem.*, 1924, **16**, 523.)—A fermented soya bean preparation, known as "miso," is one of the staple food products of Japan. It contains from 4·8 to 11·5 per cent. of liquid or semi-solid oil having the following characteristics:—Sp. gr., 0·9466 to 0·9471; acid value, 22·49 to 77·69; saponification value, 211·0 to 235·3; iodine value, 93·5 to 97·96; Hehner value, 93·53 to 97·96; Reichert-Meissl value, 3·56 to 7·46. When decolorised with charcoal, the oil becomes nearly solid, the iodine value is decreased by 30 to 40 units, the saponification value increased by 4 to 60 units, but the acid value is unaffected. W. P. S.

Analysis of Gelatin Ash. E. Cattelain. (*J. Pharm. Chim.*, 1924, **29**, 414–417.)—The composition of the mineral matter obtained from a representative commercial gelatin manufactured for pharmaceutical and bacteriological purposes, was found to be as follows:—*Soluble*: Lime, 53·36; magnesia, trace; ferric oxide, 1·22; phosphoric anhydride, 5·40; and sulphuric anhydride, 36·36 per cent. *Insoluble*: Silica, 3·36 per cent. The average composition of the ash of the purest commercial gelatins shows the sum of the lime and sulphuric anhydride to be 90 per cent., and that of the silica, iron sesquioxide and phosphoric anhydride to be 10 per cent. Traces of copper are very frequently found, whilst manganese is almost constantly present, and may, to a certain extent, influence bacteriological cultures. It may be noted that the copper was estimated colorimetrically by dissolving 1 grm. of the ash in 10 c.c. of 25 per cent. sulphuric acid, making up the solution to 20 c.c. with ammonium hydroxide solution, and comparing the colour with that shown by a solution of copper sulphate containing 1 mgrm. of copper per c.c. Manganese was also estimated colorimetrically by Bertrand's method of comparison with a standard solution of crystalline manganous sulphate, acidified with nitric acid, the ash being dissolved in 25 per cent. nitric acid, and the solutions in each case treated with 5 drops of 10 per cent. silver nitrate solution, and 0·25 grm. of potassium persulphate. The maximum amount of manganese found was 0·10 per cent. D. G. H.

Estimation of Eugenol in Clove Oil. J. A. L. Bouma. (*Pharm. Weekblad,* 1924, **61,** 249–250.)—It is shown that the factor for converting the reduced silver into eugenol in van Eck's method of estimation (ANALYST, 1923, **48,** 567) is not a constant, but varies with the amount of eugenol present and the duration of heating. Thus, by varying the conditions, factors ranging from 1·17 to 1·83 were obtained, and the conclusion is therefore drawn that the reaction is unsuitable for the quantitative estimation of eugenol.

Estimation of Santonin in Wormseed. O. P. A. Schaap. (*Pharm. Weekblad,* 1924, **61,** 277–280.)—Five grms. of the drug are dried in a desiccator and mixed with 1·5 grms. of slaked lime and sufficient water to form a paste. After standing 24 hours the mass is treated with 100 c.c. of water, and boiled for 30 minutes, after which a solution of 5·5 grms. of zinc sulphate in a little water is added, and the boiling continued for 10 minutes. The liquid is filtered, the filter and residue boiled for 15 minutes with 100 c.c. of water, and this new extract filtered. The united filtrates containing the santonin, probably in the form of zinc santoninate, are treated with 3 c.c. of 30 per cent. nitric acid and evaporated to dryness, and the residue treated with 25 c.c. of water and again evaporated. Ten c.c. of water and 2 grms. of calcium carbonate and a little sand are then added, and the mixture cooled, transferred to a separator, and shaken for 2 minutes with 50 c.c. of chloroform. The extract is filtered and 40 c.c. of the filtrate (=4 grms. of the sample) are evaporated, the residue dissolved in 5 c.c. of methyl alcohol, and the solution diluted with 35 c.c. of water at about 60° C. and left for 24 hours. The crystals of santonin are then collected on a weighed filter, washed with five successive quantities of 2 c.c. of water, and dried at 100° C. By this method practically the whole of the resin acids in the drug is eliminated.

Biochemical, Bacteriological, etc.

Alkyl-chloro-malonamides. The Influence of Homology on Taste. A. W. Dox and B. Houston. (*J. Amer. Chem. Soc.,* 1924, **46,** 1278–1281.)— All the normal alkyl-chloro-malonides have a decidedly sweet taste, the *n*-butyl derivative being both bitter and sweet. Dichloromalonamide, and ethyl-, *n*-propyl-, *iso*-propyl-, and *n*-butylchloromalonamides have about nine times the sweetening power of sucrose. The maximum sweetness was observed with the *n*-hexyl derivative, of which 1 part in 5000 of water had the sweetness of a 6 per cent. solution of sucrose. This observation was confirmed by comparison with a solution of "dulcin" (*p*-phenetyl urea), which is stated to be 250 times as sweet as sucrose. On the other hand, the *iso*-butyl, *iso*-amyl and benzyl derivatives stand in sharp contrast to the other members of the series, being decidedly bitter and without any sweet taste. No constant variation in sweetness could be established in the series. The sweet taste of the hexyl derivative is free from any disagreeable after-taste, but the compound has the drawback of being difficult to dissolve in water.

Rubidium and Caesium Creatinine Picrates. I. Greenwald and J. Gross. (*J. Biol. Chem.*, 1924, **59**, 613–614.)—Since Jaffé's creatinine potassium picrate, in spite of its slight solubility, is too soluble for use in isolating very small amounts of creatinine from large quantities of biological material, an attempt was made to prepare other double picrates of creatinine. The hot solutions of creatinine, picric acid and the sulphate or chloride of the metal or radicle were mixed in equivalent quantities, and a little sodium acetate was added. The crystals obtained on cooling were recrystallised from hot water. With lithium and sodium only creatinine picrate was obtained, and with ammonium picrate and trimethyl ammonium picrate mixtures of these with creatinine picrate were obtained. After several recrystallisations, however, pure creatinine picrate was isolated. Definite double salts were obtained with rubidium and caesium, viz. creatinine rubidium picrate and creatinine caesium picrate. Results of analyses are given. Both salts are considerably less soluble than creatinine potassium picrate, and it is proposed to use the rubidium salt, which is the least soluble of the three, in an attempt to isolate creatinine from blood.   P. H. P.

Chemistry of Jaffé's Reaction for Creatinine. I. Greenwald and J. Gross. (*J. Biol. Chem.*, 1924, **59**, 601–612.)—Little is known of the chemical changes involved in the reaction of Jaffé (*Z. physiol. Chem.*, 1886, **10**, 391) for creatinine. From experiments to determine the rate of destruction of picric acid in this reaction, which are described and results of which are tabulated, it was found that only 1 mol. of picric acid appears to be required for each mol. of creatinine, although a considerable excess (2·5 mols.) is necessary to complete the reaction. Both picric acid and creatinine may be quantitatively recovered if the alkaline mixture, made as recommended by Folin (*Z. physiol. Chem.*, 1904, **41**, 223) for a creatinine estimation, is re-acidified within 10 minutes, but after 30 minutes the former can no longer be quantitatively recovered, since the colour of the alkaline mixture begins to fade. A red precipitate was obtained on adding hydrochloric acid to a concentrated mixture of creatinine, excess of sodium picrate and slight excess of sodium hydroxide. After washing and drying this formed a brilliant red powder which is, apparently, a tautomeric form of creatinine picrate, since it became creatinine picrate at 139° C. The formation of this substance appears to be responsible for the red colour in Jaffé's reaction for creatinine, which Chapman (ANALYST, 1909, **34**, 475) thought was due to the formation of the sodium salts of picramic acid (monoamino-dinitrophenol) and diamino-nitrophenol. Certain other substances, similar in constitution to picric acid, did not give the reaction. A slight intensification of the colours of alkaline solutions of the salts of trinitrobenzene, trinitrotoluene and trinitrobenzoic acid was observed.
                                        P. H. P.

Estimation of Histamine. M. T. Hanke and K. K. Koessler. (*J. Biol. Chem.*, 1924, **59**, 879–888.)—The work reported in Papers XVII. and XVIII. of this series (*J. Biol. Chem.*, 1924, **59**, 835 and 855) brings definite proof that the normal bacterial inhabitants of the alimentary tract have the faculty of converting

the innocuous amino acids, histidine and tyrosine, into the highly toxic amines, histamine and tyramine.   A purely chemical method is described for the estimation of histamine in the liver, intestinal contents, and faeces of animals.   The material to be examined for histamine is hydrolysed by heating with 37 per cent. hydrochloric acid for 30 hours, and the black insoluble residue formed is filtered off.   The acid is removed by distillation *in vacuo* at 60° C. from the same flask.   The residue is then dissolved in water, and the mixture is treated with excess of lime and a volume of alcohol equal to one-half the volume of water added.   The mixture is distilled *in vacuo*, whereby the ammonia is removed, and filtered to remove the humin.   The colouring matter of the alkaline solution is progressively lost during the subsequent steps of the process, but, if not completely removed, mercuric sulphide precipitated in the solution at the end adsorbs it.   The alkaline filtrate is acidified with hydrochloric acid, concentrated on a water-bath, and treated with phosphotungstic acid in acid solution.   The phosphotungstates are removed by filtration, and the washed precipitate, containing the histamine, is macerated in a mortar with a hot, concentrated barium hydroxide solution, heated on a water-bath for 1 hour, filtered and the barium removed from the filtrate with sulphuric acid.   The acid filtrate is concentrated on the water-bath, the residue diluted with water, made alkaline, and extracted exhaustively with amyl alcohol, which removes histamine quantitatively.   The alcohol extracts are extracted five times with N sulphuric acid; the histamine passes into the aqueous acid layer, which is neutralised with barium hydroxide, filtered and concentrated; the residue is dissolved in water, and the solution made alkaline and extracted with amyl alcohol as above.   This is done three times in all, and is very important.   The final residue is dissolved in water and estimated colorimetrically (*J. Biol. Chem.*, 1919, **39**, 597). Human faeces (500 to 600 grms. from normal individuals) have yielded from 6 to 20 mgrms. of histamine.   The caecal content (600 and 1200 c.c.) contained 2 and 7 mgrms. of histamine respectively.   One human liver examined yielded none. Dog faeces (150 grms.) contained 5·3 mgrms., and the liver of the same dog yielded 6 mgrms. of histamine.   The intestinal contents, intestinal tract, and liver of two normal guinea pigs did not yield histamine.   P. H. P.

Use of Guaiacol for Measuring the activity of Oxidase Preparations. P. Fleury. (*J. Pharm. Chim.*, 1924, **29**, 402–414.)—Under certain conditions guaiacoquinone, a product of oxidation of guaiacol, can be estimated colorimetrically, the oxidation of guaiacol in the presence of air and laccase being very nearly complete, but the yield is slightly variable, possibly owing to the decoloration of guaiacoquinone solutions by excess of guaiacol.   The laccase was found to be practically unaltered at the end of the reaction.   Since, however, the colouring matter tends to precipitate and cannot be redissolved in excess of water, owing to the formation of a colloidal solution, it must be separated before any estimation can be made ; for this purpose chloroform is used, as it at once removes practically the whole of the guaiacoquinone and renders the reaction more stable.   It was found that a solution of 46·5 mgrs. of guaiacoquinone in a litre of chloroform had

the same tint as a $N/100$ solution of iodine observed through a thickness of 10 mm. The chloroform solution must be prepared by directly extracting the water solution of guaiacol treated with laccase, and attention must be paid to the relation between the proportion of guaiacoquinone and the time of contact of the colloidal solution of guaiacoquinone with the guaiacol. For details of working, the original paper should be consulted. D. G. H.

Influence of the Bactericidal Power of Raw Milk on Lactic Acid Organisms in Sterilised Milk. P. Mazé. (*Comptes rend.*, 1924, **178**, 1434–1436.) —Occasionally in the spring, and less frequently in the autumn, lactic acid organisms which have been kept in sterile milk lose their power of acidifying pasteurised skim milk or cream. Apparently the bactericidal power of the fresh milk undergoes reinforcement owing to the change of the cows' diet. T. H. P.

Toxicological and Forensic.

**A Case of Mercuric Chloride Poisoning. Limit of Sensitiveness of the test for Mercury in Toxicology.** A. Sartori. (*Chem. Zeit.*, 1924, **48**, 141–142.) —An unusual and fatal case of corrosive sublimate poisoning is described which was due to the use of a very weak solution of this salt as a vaginal wash. Subsequent examination of parts of the body by the usual method of the destruction of organic matter by potassium chlorate and hydrochloric acid, followed by hydrogen sulphide precipitation, yielded negative results, even when working on quite large quantities of the material. Similar tests on the intestines and kidneys showed only a trace of mercury. Experiments made show that the most delicate test is the deposition of the mercury on copper foil and the preparation therefrom of mercuric iodide in the manner recommended by Jannasch (*Zeitsch. anorg. Chem.*, **12**, 43). This will detect 0·05 mgrm. in aqueous solution, or about 2 mgrms. in admixture with organic matter. H. E. C.

Spectroscopic Identification of Mercury. Naoum. (*Chem. Zeit.*, 1924, **48**, 311).—Quantities of mercury too small for detection by chemical methods may be identified spectroscopically. The substance in form of a dry powder is placed in the two limbs of the tube figured, which is then exhausted at the pump. The limbs are heated in a water-bath and, on passing a current through the vacuum tube thus produced, the mercury spectrum may be identified in the cross piece (*c*). Still greater delicacy is obtained by subliming the mercury on to pure gold-leaf, which readily absorbs it; the gold-leaf is then examined spectroscopically in the above manner.

H. E. C.
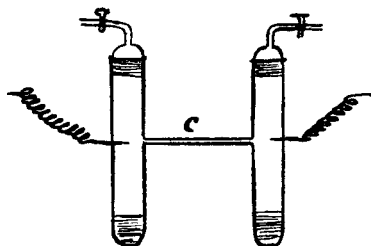
Organic Analysis.

Oxalic Acid as a Standard Substance for Titrations. W. D. Treadwell and H. Johner. (*Helv. Chim. Acta*, 1924, **7**, 528–534.)—Anhydrous oxalic acid is difficult to obtain and is too hygroscopic for general use; the ordinary hydrated acid contains occluded water, which makes it useless as a standard substance. The vapour pressure curve has been investigated by the dew-point method and the equilibrium is worked out in detail. For purification, the acid is recrystallised from hydrochloric acid of sp. gr. 1·07, as directed by Winkler, but the method of drying given by Lunge (6 hours at 70–80° C.) is not satisfactory. The anhydrous acid can be obtained pure by the method first given by Hampe (*Chem. Zeit.*, 1883, **7**, 73, 106), which consists in drying the crystals at 100° C., then subliming them *in vacuo* at 140° C., again drying them at 100° C., and finally cooling them in a desiccator over calcium chloride. For analytical purposes the hydrated acid is best; it may be prepared by passing air first through a large wash bottle containing equal parts of hydrated and of anhydrous acid, and then through a small weighed tube containing the powdered crystals of the hydrated acid until this is entirely free from occluded water, as is shown by the weight becoming constant. H. E. C.

Estimation of Organic Phosphorus. E. J. Baumann. (*J. Biol. Chem.*, 1924, **59**, 667–674.)—Experiments described shew that serious losses of phosphorus may occur in the wet ashing used in the micro-methods for estimating phosphorus, these being due to (*a*) Volatilisation of phosphoric acid; and (*b*) conversion of ortho-phosphoric to meta- and pyrophosphoric acids. A process has been developed to avoid these losses, and it allows from 0·02 mgrm. to several mgrms. of phosphorus to be estimated accurately and easily. By heating with 20 to 30 per cent. hydrogen peroxide and sulphuric acid, organic matter oxidisable with difficulty can be oxidised in a short time, and at a comparatively low temperature, sufficiently to liberate all combined phosphorus as orthophosphoric acid. Free saturated acids formed from fats do not interfere in the estimation. Applications of the method are described for estimations of lipoid phosphorus in animal tissues and fluids by using the Bloor alcohol and ether extraction process, oxidising with hydrogen peroxide and sulphuric acid, and finally estimating the phosphorus by the colorimetric method of Bell and Doisy (*J. Biol. Chem.*, 1920, **54**, 55). The tissue is ground to a paste, a small sample weighed out, mixed with plaster of Paris and dried over sulphuric acid in a vacuum desiccator. Then it is thoroughly pulverised in a mortar, with the aid of washed powdered glass, and transferred to a Gooch crucible on an asbestos mat. The substance is next extracted in a Wiley extraction apparatus with anhydrous ether during the day (6 hours), and with anhydrous alcohol overnight. This process is repeated during the next 24 hours, and, finally, the substance is extracted a third day with alcohol. In this 3 day-extraction, 95 per cent. of the material soluble in alcohol-ether which can be extracted in 10 days is obtained. It is concentrated to a few c.c. and dried overnight in the vacuum dessicator. The fatty material is dissolved in anhydrous chloroform, filtered several times (in

case plaster of Paris is still present), evaporated nearly to dryness, dried in a vacuum dessicator over sulphuric acid for 15 hours and weighed, then re-dissolved in a definite amount of chloroform ready for the estimation.   All the apparatus used is washed with the various solvents or extracted.   The greatest source of error lies in the extraction processes.   The same process as for lipoid phosphorus estimations of blood, full details of which are given, is then employed, but usually more hydrogen peroxide is needed for the oxidation.   P .H. P.

Testing Decolorising Carbons.   J. E. Temple and P. Mahler.   (*Ind. Eng. Chem.*, 1924, 16, 498–500.)—With a Hess-Ives tint photometer and a given solution, the action of any carbon on that solution may be represented on logarithmic paper by a straight line, and the comparative value of different carbons acting on the same solution may be easily deduced.   It is usually sufficient to follow the decolorisation only with the light which is most strongly absorbed; in the case of many commercial liquids this is the blue-violet, since they are usually coloured red, yellow or brown and absorb but little red or yellow light.   Of course, when decolorising a blue-violet solution, absorption of red or green light would be measured.   The action of carbons on one solution cannot be applied to another solution; tests made on iodine solution or by other so-called standard methods were of little practical value, and the method of simply recording the decolorising power on any given solution as 70 or 90 is misleading.   The adsorption of colour by carbon is an equilibrium reaction, but substances other than colouring matters assist in determining the equilibrium values.   W. P. S.

Estimation of Fat, Wax and Resin in Cotton.   P. H. Clifford, L. Higginbotham and R. G. Fargher.   (*J. Textile Inst.*, 1924, 15, T 120.)—Solvents extract from cotton, fat, fatty acids, wax-alcohols, acids and esters, phytosterol, phytosterol glucoside, hydrocarbons, resin acids and resenes, so that it is impossible to eliminate one group and leave the others unaffected.   Experiments have been carried out for varying periods of time with the use of different solvents, such as chloroform, benzene, carbon tetrachloride, ether, petroleum spirit, etc. The Soxhlet apparatus is made of tinned copper large enough for 100 grms. of cotton and 1200 c.c. of solvent, and is of two forms:—(1) For extracting the material at the ordinary temperature; (2) for extracting the material at the boiling point of the solvent.   The drying of the extract is recommended to be carried out at 15 mm. pressure and 80° C., rather than at 760 mm. and at 100 to 110° C., as no darkening occurs, and the method is quicker.   To obtain uniform results rigid conditions must be maintained.   For fat, wax and resin, chloroform should be used in the hot Soxhlet for 6 hours.   For fat and wax only, carbon tetrachloride should be used in an ordinary Soxhlet extractor.   The presence of size does not materially interfere with the removal of the natural wax and resin. For large-scale extractions, chlorinated solvents should be avoided, since they are susceptible to hydrolysis, with liberation of free hydrochloric acid which might tender the cotton when steaming off the last traces of solvent after extraction.

                              R. F. I.

Chrome-leather Analysis. D. Woodroffe. (*J. Soc. Leather Trades Chem.,* 1924, **8**, 194.)—In the estimation of moisture, a recommendation is made to use air-tight aluminium boxes, $3\frac{1}{2}$ ins. diam. $\times \frac{1}{2}$ in. deep, in which the leather can be weighed out, dried, transferred to the desiccator and re-weighed, all in the same vessel. These are preferable to weighing-bottles. On the question of fat-estimation the author has done some work on the use of different solvents in succession, and also on the effect of drying the leather before extraction. He recommends that the leather be dried for 2 to 3 hours at 100–110° C. and extracted with petroleum spirit for 3 hours. The solvent (b.pt. 40–60° C.) should siphon over at least twelve times. The extract is evaporated and dried in a water-oven for 2 hours, or to constant weight. *Basicity of the leather:* The method for this estimation as carried out by Meunier and Chambard is compared with the older method, in which the SO_4-radicle is estimated by treatment of the dried ether-extracted and water-extracted leather with fuming nitric acid in the cold for three days. Experiments are described which tend to show that Meunier and Chambard's method is only satisfactory for freshly-tanned wet leather, and not for finished dried leather. Results are given in terms of the new official form of expression (per cent. chromium combined with hydroxyl), and also the older English method (grms. of SO_4 per 52 grms. of chromium). A method for estimating alumina is quoted, in which aluminium and chromium are estimated together gravimetrically, and the aluminium calculated after subtracting the chromium as estimated volumetrically. Results are said to be too high, owing to salts being precipitated with the hydroxides. R. F. I.

Standard Methods for the Analysis of Rubber Articles. (*Ind. Eng. Chem.,* 1924, **16**, 397–402.)—The following methods of analysis are recommended by the Committee on Methods of Analysis of the Division of Rubber Chemistry of the American Chemical Society. *Acetone Extract.*—Two grms. of the sample, ground to pass a No. 14 sieve or cut into small pieces, are extracted in a siphon apparatus for four hours, or for twelve hours in the case of hard rubber; the acetone solution is then evaporated, the residue dried at 70° C., and weighed. From vulcanised material the acetone removes rubber resins, free sulphur, mineral oils and waxes, and a part of any bituminous substances of vulcanised oils that may have been used. The free sulphur is estimated by oxidation with bromine and its quantity deducted from the total extract; the corrected extract thus obtained should not exceed 5 per cent. of the rubber present. *Chloroform extract.*—The sample of rubber, without removal of adhering acetone, is extracted for four hours with chloroform, the chloroform solution is evaporated, and the residue dried at 70° C., and weighed; this residue consists of a portion of the bituminous substances and serves as an indication of their presence. *Alcoholic alkali extract.*—The rubber after the two previous extractions is dried and boiled with 50 c.c. of N alcoholic sodium hydroxide solution for four hours under a reflux condenser, the solution is then filtered, the rubber washed with hot alcohol and then with water, the filtrate is evaporated to dryness, the residue mixed with water, acidified with hydrochloric

acid and extracted with ether; the residue obtained on evaporating the ethereal solution is dried and weighed; it represents the amount of rubber substitutes present. *Total sulphur.*—This is estimated by treating 0·5 grm. of the rubber with 15 c.c. of nitric acid saturated with bromine for one hour, heating the mixture on a water-bath one hour and evaporating it to dryness; the residue is mixed with 3 c.c. of nitric acid and 5 grms. of sodium carbonate, dried, fused, and the sulphate estimated by precipitation as barium sulphate. An alternative method is given for the estimation of total sulphur other than that present as barium sulphate, if this has been used as a filler; the sample is treated with a mixture of zinc oxide and nitric acid (prepared by adding 200 grms. of zinc oxide to 1 litre of nitric acid), oxidised with fuming nitric acid and bromine, the mixture evaporated to dryness, the residue baked, dissolved in hydrochloric acid, the solution filtered and the sulphate estimated in the filtrate. *Ash.*—The ash of the sample should be estimated; it represents the non-volatile fillers together with their reaction products with sulphur. *Rubber.*—This is estimated indirectly after allowance has been made for carbonates, cellulose, and hydrocarbons of mineral rubbers. Special methods are also given for the analysis of materials which contain glue, carbon, antimony and waxy hydrocarbons.   W. P. S.

Inorganic Analysis.

Precipitation of Zinc as Sulphide. O. Hackl. (*Chem. Zeit.*, 1924, **48**, 326.)—In Schneider's method for the precipitation of zinc sulphide from feebly acid (sulphuric) solutions, proper neutralisation is a decisive factor for the success of the operation. The solution, to which a piece of Congo paper is added as indicator, should not be too dilute; it is made distinctly ammoniacal, acidified with sulphuric acid, then treated with strong ammonia solution, drop by drop, until the alkaline reaction persists after 5 to 10 seconds' stirring. The prescribed small excess of sulphuric acid, calculated on the subsequent dilution desired, is then added.   W. R. S.

Volumetric Assay of Iron Ores by means of Titanous Chloride. L. Brandt. (*Chem. Zeit.*, 1924, **48**, 265–266, 270–271.)—The titanous chloride method of Knecht and Hibbert possesses the advantage over the usual volumetric methods of not requiring preliminary reduction of the iron to the ferrous state. It was tested as to its applicability to the assay of iron ores, the tests bearing on the uniformity and proportionality of the results and the question of the interference of other elements. Thiocyanate was used as internal indicator, being added either at the outset or after the bulk of the ferric salt had been reduced. The titrations were carried out at ordinary temperatures; it was found unnecessary to maintain an atmosphere of inert gas over the solution undergoing titration, but the stock solution of titanous chloride must be stored in a bottle communicating with a source of carbon dioxide; in this manner it kept unchanged for several weeks. The process was found to be simple and accurate, and to give almost

exactly proportional results. The only interfering metals are copper, antimony, vanadium, and platinum, whilst larger quantities of chromium and cobalt affect the end-point more or less by yielding coloured solutions. The interference of platinum (derived from the crucible in the case of a bisulphate fusion) is smaller than in the permanganate titration. Antimony, cobalt, and vanadium occur very rarely and in negligible quantities; for practical purposes, copper is the only metal which seriously interferes, being quantitatively reduced to the cuprous state.

<div align="right">W. R. S.</div>

Detection of Cobalt and Nickel. C. C. Palit. (*Chem. News*, 1924, **128,** 293–294.)—A few drops of the solution are added to a saturated solution of sodium bicarbonate. The cold liquid is shaken with a little bromine water; an apple-green colour indicates presence of cobalt. The solution is then heated, when a bluish-black precipitate (forming a filmy deposit on the glass) shows the presence of nickel.

<div align="right">W. R. S.</div>

Analysis of Sodium Peroxide. E. Bosshard and E. Furrer. (*Helv. Chim. Acta*, 1924, **7,** 486–489.)—Two methods are available for the estimation of the active oxygen in sodium peroxide, *viz.* gasometric and volumetric. For the former 0·2 to 0·6 grm. of the powder, weighed in a closed tube, is introduced into a small flask fitted with a fine dropping funnel and connected with a Bunte burette; 15 c.c. of 1:10 sulphuric acid and 3 drops of a saturated solution of cobalt nitrate are slowly added from the dropping funnel, and afterwards the flask is gently warmed. The gas evolved may be measured over brine or potassium hydroxide solution, the latter having the advantage of absorbing any carbon dioxide. The difficulty with the permanganate titration method is the decomposition brought about by the heat of hydration of the sodium peroxide; this may be overcome by the following method, which gives results identical with those of the gasometric method:—From 0·2 to 0·4 grm. of the peroxide is mixed in a mortar with 3 to 5 grms. of powdered boric acid. Then 100 c.c. of water and 10 c.c. of 1:5 sulphuric acid are added, and the resultant hydrogen peroxide is titrated in the usual manner with 0·1 N potassium permanganate solution.

<div align="right">H. E. C.</div>

Detection of Phosphoric Acid. F. Frey. (*Chem. Zeit.*, 1924, **48,** 281.)— Feigl's benzidine reaction (ANALYST, 1923, **47,** 93) was found to be easily carried out and extremely sensitive, as it indicated less than 0·000,001 grm. of P_2O_5 in 0·1 c.c. A small quantity of the solution is acidified with nitric acid and treated with twice its volume of the usual molybdate reagent. The solution is warmed for a short time, and run through a filter paper of close texture. A drop of ammonia solution is brought into the apex of the filter by means of a glass rod, and a drop of benzidine acetate solution poured upon the paper, when the intense blue coloration is produced if phosphoric acid is present. The reaction has the additional advantage that the yellow precipitate of molybdic acid, which sometimes forms, cannot be mistaken for ammonium phosphomolybdate.

<div align="right">W. R. S.</div>

Physical Methods, Apparatus, etc.

Simple Method for determining the Approximate Index of Refraction of Liquids with a Common Microscope. C. C. Kiplinger. (*J. Chem. Soc.*, 1924, **125**, 963–965.)—A Johannsen auxiliary lens may act as a telescopic objective, the optical system of the microscope becoming the eyepiece. If such a spherical lens is placed above, and in contact with, a glass slide on the stage, the lens being brought under the objective and the whole system focussed on an object one or two feet from the microscope, the introduction of a drop of liquid between the lens and the slide changes the focus of the optical system by an amount proportional to the index of refraction of the liquid.

The auxiliary lens is made by drawing out a soft glass rod to a diameter of about 0·3 mm. and then fusing a bead on the end of a 4 cm. length of the fine rod, any bead failing to give a sharp image being discarded. A bead 1·81 mm. in diameter gave satisfactory results with a 7·5 eyepiece and a 32 mm. objective. If the stem of the bead lens is bent at right angles a few mm. from the spherical tip, the same side of the lens may always be brought next to the glass slide by using the bent stem as an index; in this way the accuracy of the determinations is greatly increased. The focal adjustment is made by moving the graduated draw-tube containing the eye-piece. The sodium lamp of Fales and Morrell (ANALYST, 1922, **47**, 40), improved by replacing the crucible by an alundum cone, is placed 30 to 40 cm. from the microscope, and between the latter and the lamp (to serve as "distant object") is a capillary glass tube, supported vertically on a large cork as base. This object is focussed and the reading on the scale taken. A drop of water is then placed between the slide and the lens, care being taken to keep the water from the upper half of the sphere; the system is then re-focussed by moving the draw-tube only, this being repeated and the mean of the several scale readings taken. The water is then replaced by glycerol and similar readings are taken. From the known refractive indices for water and glycerol, and the reading given by an unknown liquid, the index of the latter may be calculated by interpolation. Results accurate to 0·02 are obtainable in this way.   T. H. P.

Receiving Apparatus for Distillation at Low Pressures. W. F. Seyer. (*J. Amer. Chem. Soc.*, 1924, **46**, 1209–1210.)—This apparatus enables a continuous fractional distillation to be made at low pressures without risk of leakage. The tube from the condenser passes through the wall of a large vertical tube having the form of a Liebig condenser, and delivers into a funnel held in position within the large tube by two metal plates supported by constrictions in the wall of the tube. The end of the stem of the funnel is inclined at an angle, so that it can be brought over any one of the openings of a series of tubes sealed into the apparatus, and, in turn, sealed to flasks the sizes of which depend upon the amounts of the various fractions. Clamped to the funnel are two small pieces of iron rod held together at the ends by brass strips, and the funnel, with the fixtures, turns on a

glass bearing which rests on a small indentation, so that it swings about easily when a magnet is brought near to either of the iron pieces. The whole apparatus is made of pyrex glass, so that it can be heated whenever there is a possibility of fractions solidifying and blocking the funnel.

Laboratory Stirrer. C. E. Waters. (*Ind. Eng. Chem.*, 1924, **16**, 493).— The stirrer described is operated by compressed air. A fan made of thin brass sheet and having twelve vertical vanes is fitted to the top of a short vertical steel shaft provided with bearings, and the lower end of this shaft is cemented on to a vertical glass tube forming the actual stirring rod. Small paddles, short closed side tubes, or the like may be attached to the bottom of this tube to aid the stirring action. Compressed air is blown on to the vanes from jets on a curved glass tube which is fixed horizontally and close to the outer edges of the vanes. W. P. S.

Reviews.

TECHNICAL METHODS OF CHEMICAL ANALYSIS. LUNGE AND KEANE. Second Edition. Edited by Charles A. Keane, D.Sc., Ph.D., and P. C. L. Thorne. M.A., M.Sc. Pp. xx.+702. London: Gurney & Jackson. Price 63s.

The first volume of the new edition of this work, which will be published in six separate volumes, has recently appeared. As stated in the preface, the volume under review contains two new sections, viz. " Electrolytic Methods of Chemical Analysis" and " Physical Methods employed in Chemical Analysis."

Section (1) of the new edition, " General Methods used in Chemical Analysis," written by Dr. Keane, is devoted mainly to the consideration of the fundamental operations of analytical chemistry. Whilst containing much that is common to all text books of analytical chemistry, the chapter is well and clearly written, but there are a few minor points which would bear revision. The description and illustration of a druggist's hand scales on p. 14 might well have been omitted, and one would have thought that the Gooch crucible was so well known as to need no description in a book written for chemists. In referring to the titration of boric acid on p. 43, the writer conveys the impression that glycerol produces a change in the indicator rather than in the acid, which is contrary to the generally accepted view. The statement occurs on p. 57 that "it is difficult to prepare a normal solution of barium hydroxide"; this compound is not sufficiently soluble in water at ordinary temperature to enable a *normal* solution to be made.

Of the new sections, that on " Electrolytic Methods of Chemical Analysis" is written by Dr. H. J. S. Sand, an original worker and acknowledged authority in this field. In the author's words, "this section consists of an account of the methods available for electrolytic analysis, of the apparatus employed, and the general conditions applicable to the solutions in which the electrolysis is effected." This section is a model of brevity and conciseness, and it is hoped that Dr. Sand will contribute further sections dealing with individual estimations.

The section on "Physical Methods employed in Technical Analysis," by Dr. J. S. G. Thomas, gives an account of many of the more important instruments employed in technical laboratories, and contains a great deal of valuable information which is not readily available to the majority of chemists. No mention is made of the spectrometer but, presumably, this will be included in a later volume.

The joint editors deal very fully with "Technical Gas Analysis," and the sampling and analysis of fuel is described by Mr. G. N. Huntly, who shows a first-hand knowledge of the subject treated.

The description of the methods used in the examination of the raw materials, products, and bye-products of the acid, alkali and chlorine industries, originally written by the late Dr. Lunge, has been revised for this edition by Dr. Dunn. Whilst acknowledging the debt which chemists owe to Dr. Dunn for the revision of this important section, the reviewer must confess to a feeling of disappointment in that the personal note is entirely lacking. A great variety of methods is included, but in far too many cases no opinion is expressed by the writer as to the value of the process described. It is noted that in the description of the determination of nitrate by reduction to ammonia and subsequent distillation the ammonia is collected in water, standard acid being added as the distillation proceeds. Surely it is more convenient to distil into excess of standard acid. In the description of the estimation of potash in saltpetre the perchlorate method is not mentioned.

The subject matter under the heading of pyrites includes the original Lunge's method together with others, and notably that of Allen and Bishop, whose method is extensively used in America. This latter method takes into account the influence of rate of addition of barium chloride upon occluded salts in barium sulphate, and is novel inasmuch as the initial attack is varied, whilst the final precipitation is made in the presence of iron in the reduced state. It is, however, erroneous to suggest that attack by means of nitric acid and potassium chlorate is accompanied by separation of sulphur. The methods given for the estimation of arsenic in pyrites include several which may, we hope without offence, be described as obsolete. The fusion method of Blattner and Brasseur yields only 80 per cent. of arsenic present, the missing 20 per cent. remaining in the residue after fusion; moreover, this method is not sufficiently delicate to yield accurate results when applied to minerals containing small amounts of arsenic. Undoubtedly the distillation method is the best available, but this cannot be considered to be presented in the most helpful fashion, for a much more simple preliminary treatment before distillation is now used in many technical laboratories. References to the work of Brand, Jannasch and Fenner would be of value in this connection.

A short description of pyrrhotite is given and could be well extended, together with references to work upon marcasite and pyrite, as the two former are frequently encountered in commercial deliveries of pyrites, and have significance as regards weathering in storage.

In the concluding section, Mr. H. Williams deals with the detection and estimation of cyanogen compounds in the various commercial products in which these occur. It is stated in the text that the methods selected are those which

combine speed with accuracy, and the author appears to have succeeded in making a wise and discriminating choice. "Lunge" has always been regarded as a standard text-book upon the subject matter treated, but, with the present additions and revision, the book now becomes practically indispensable to all chemists who are engaged upon the general scope of the work; accuracy of description of methods is carried to a high degree and the new volume is to be recommended as worthy of being on the reference shelves of chemists, manufacturers and others who have to consider the subject from the technical aspect.

The indexing is ample and the references, on the whole, comprehensive.

G. RUDD THOMPSON.

X-RAYS. THEIR ORIGIN, DOSAGE AND PRACTICAL APPLICATION. By W. E. SCHALL. Pp. 119. Bristol: John Wright & Sons, Ltd. 1923. Price 5s.

It is common knowledge that X-ray therapy is frequently as efficacious as, and certainly preferable to a major operation for the treatment of certain diseases involving tissue degeneration. Probably thousands of exposures are made daily for diagnostic or curative purposes, so it is not surprising that severe injuries have occasionally been caused. Most of these are due to over-exposure or to excessive dosage. In view of the growing importance of the subject, the appearance of this little book, which bears evidence of having been written by one who has an extensive practical knowledge of the subject, must be welcomed. The author outlines in a simple way the theory of X-rays, then describes in fair detail the different kinds of tubes and other accessories required in connection with their application. The very important question of the quality of the rays, their measurement and dosage for diagnostic or therapeutic purposes is discussed in detail.

The reviewer ventures to call attention to two points which would increase the usefulness of the book in any subsequent edition; one is that, although the work of various investigators and their names are freely mentioned, in most instances no references are given to the books or journals in which the original papers may be consulted. This is of importance, especially in connection with certain methods of measurement for which full working details are necessary. The other point is that an index should be added; a table of contents at the beginning is by no means adequate for rapid reference.

H. E. Cox.

TESTED METHODS OF MINERAL ANALYSIS. B. T. KITTO. Pp. 127. London: H. F. & G. Witherby. 1924. Price 7s. 6d. net.

A Foreword by Mr. Benedict Kitto states that the methods described in the present work are those that have been tested and found to give accurate results in his own laboratory, where the author has had twenty years' experience. The book is of a type with which, of late, metallurgical chemists have become rather familiar: it describes, in language of a rich local flavour and without any references to text-books or original papers, the routine followed in a particular laboratory.

In too many cases the chemical change occurring as the result of the operation prescribed is left unexplained, with the result that the less experienced operator has to follow such directions without understanding their bearing on the progress of the estimation.

The methods include a number that may now be considered out of date, while several are rather questionable. The following are examples of obsolescent processes. The nitrite method is used for the separation of nickel from cobalt, without the choice of an alternative. Dimethylglyoxime, nitroso-β-naphthol, and the volumetric cyanide method for nickel are not mentioned; nickel is estimated in the filtrate from the potassium cobaltinitrite precipitate by precipitation with potassium hydroxide (in a glass vessel) and ignition to oxide; no purification of the oxide from silica and adsorbed potassium salt is carried out. Similarly, the only "tested" method for total manganese is treatment of the filtrate from the basic acetate precipitation with sodium carbonate and ignition of the precipitate to Mn_3O_4, without subsequent purification. For the estimation of arsenic, the sulphide is converted into arsenic acid and the latter titrated with uranium acetate, ferrocyanide being the external indicator. With two iodimetric methods and an acidimetric method at his disposal, the operator can afford to discard his spot-plate when titrating for arsenic. The magnesium assay is carried out by titration of the magnesium ammonium phosphate with the same uranium solution as is used for arsenic; the magnesium value of the solution is obtained by calculation from the arsenic factor, clearly an error of judgment, as the end-point does not strictly correspond to the stoichiometric reaction. Sutton, one of the originators of the uranium method, insists that the standardisation should be done under exactly the same conditions as the assay. Cadmium is weighed as sulphide on a tared filter, by no means the best method; incidentally, the quantity of zinc ore taken is one grm., and the small precipitate of cadmium sulphide obtained therefrom collected on a 12·5 cm. filter; this tendency to use unduly large filter papers is fairly general throughout the book. For small quantities of titanium, the obsolete procedure of hydrolysing the dilute sulphuric acid solution is still recommended, despite the universal adoption of Weller's colorimetric method. As regards silica estimations, no mention is made of the final purity test by evaporation with hydrofluoric acid.

Hæmatite is wrongly credited with giving an iron-black streak (p. 54). Misprints are not numerous and are quite obvious, e.g. the long ton is stated to contain 32·6 ounces (p. 49).

In conclusion, the writer believes he voices the feelings of the great majority of his colleagues when he deplores the careless and loose style, the disregard of grammatical rules, and the use of slang in a growing number of technical books.

W. R. SCHOELLER.

THE VEGETABLE PROTEINS. By THOMAS B. OSBORNE, Ph.D., Sc.D. (Monographs on Biochemistry.) Pp. xiii. +154. London: Longmans, Green & Co. 1924. Price 9s. net.

Dr. Osborne's work on the vegetable proteins has gained him international repute, and we must welcome the appearance of the collective summary of his many researches on this very difficult subject. This book is obviously written by a specialist, and, as such, it has all the advantages and disadvantages of books which are written by highly specialised authors. The advantages of this book are many, but the disadvantages too are also striking. Thus about thirty years ago Osborne set out to disprove Ritthausen's theory that there is but one vegetable protein common to all plants. Since then Ritthausen has died and Osborne has become the recognised authority on plant proteins. In spite of this, Osborne continues to tilt against Ritthausen. The present state of the chemistry of the vegetable proteins reminds one of the chemistry of chlorophyll. Here, too, were two opposing schools which held that there were as many chlorophylls as plants, or that all plants contained the same chlorophyll respectively. This latter view has now been established beyond doubt by the classical researches of Willstätter. Is it therefore not possible that the plant proteins may some day also have their Willstätter to unite them?

English readers, for whom the Monographs on Biochemistry are, after all, mainly intended, will be disappointed to find that Osborne uses the American protein nomenclature. But still more bewildering is the fact that the "hæmoglobins" are classed among the "vegetable proteins" (p. 17). Similarly surprising is the statement that the protein from *Ceramium rubrum* belongs to the "hæmoglobins," since it "contains some derivative of *flavone*" (p. 32).

However, most disappointing is the incompleteness of the bibliography, although it covers 28 pages. Such important work as Guggenheim's on the protein and the amino acids of *Vicia faba* has been omitted, whereas three papers by Herzig on the methylation of several *animal* proteins with diazomethane are included. If it is intended to make special reference to this method of methylation of proteins, then, again, why have the publications of Biltz, Johnson and others been omitted? They also deal with the action of diazomethane on proteins and amino acids.

Reference must also be made to the omission of Zelinsky's work on the constitution of the proteins. It is true Zelinsky's results are fundamentally opposed to the now generally accepted concepts of Emil Fischer. But are we to be lulled into the belief that Emil Fischer was infallible?   M. NIERENSTEIN.

REPORTS OF THE PROGRESS OF APPLIED CHEMISTRY ISSUED BY THE SOCIETY OF CHEMICAL INDUSTRY. Vol. VIII. Pp. 600. 1923. Price 7s. 6d. to members. 12s. 6d. to non-members.

In this exceedingly useful annual the sections are similar to those of previous issues, but the section on photographic processes is now omitted. No reason is

given for the omission.   Most chemists interested in photography will regret the loss of this section.

Marked advance is shown on the year's work in all branches of chemistry.

The section on Foods has again been written by Dr. G. W. Monier-Williams, and much of the analytical work on this subject published during the year will be found summarised here, as well as the work on Vitamins and Milk and Dairy Products.   Other sections of interest to the public analyst are those on Oils, Fats, Waxes, by R. J. Pelly; Soils and Fertilisers, by H. J. Page; the Fermentation Industries, by H. L. Hind; and Sugar, Starch and Gums, by R. F. Lyle.

These annual volumes are indispensable to every chemist, and are worth possessing, if only for the full bibliographies under each section.

<div align="right">R. Leitch Morris.</div>

Publications Received.

CHEMICAL SYNTHESIS: STUDIES IN THE INVESTIGATION OF NATURAL ORGANIC
PRODUCTS. By HARRY HEPWORTH. Pp. xx. + 244. London: Blackie &
Son, Ltd. 1924. Price 20s. net.

CHEMICAL ENCYCLOPAEDIA. By C. T. KINGZETT. Pp. x. + 606. London:
Baillière, Tindall & Cox. 1924. Price 30s. net.

QUALITATIVE ORGANIC ANALYSIS. By OLIVER KAMM. Pp. 260. New York:
John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. Price 12s. 6d. net.

TISSUE CULTURE IN RELATION TO GROWTH AND DIFFERENTIATION. By T. S. P.
STRANGEWAYS. Pp. 50. Cambridge: W. Heffer & Sons, Ltd. 1924.
Price 5s. net.

THE TECHNIQUE OF TISSUE CULTURE "IN VITRO." By T. S. P. STRANGEWAYS.
Pp. 80. Cambridge: W. Heffer & Sons, Ltd. 1924. Price 7s. 6d. net.

THE SPECIFIC HEAT OF GASES. By J. R. PARTINGTON and W. G. SHILLING.
Pp. 252. London: Ernest Benn, Ltd. 1924. Price 30s. net.

CHEMISTRY IN THE TWENTIETH CENTURY. Edited by Dr. E. F. ARMSTRONG.
Pp. 281. London: Ernest Benn, Ltd. 1924. Price 15s. net.

CHEMICALS. By A. W. ASHE and H. G. J. BOORMAN. Pp. 207. London: Ernest
Benn, Ltd. 1924.

LIME AND MAGNESIA. By N. V. S. KNIBBS. Pp. 306. London: Ernest Benn
Ltd. 1924. Price 30s. net.

CHEMICAL INDUSTRY PAMPHLETS:
> THE QUEST FOR COLOUR. By A. T. DE MOUILPIED. Pp. 31.
> CHEMISTS AND THEIR WORK. By STEPHEN MIALL. Pp. 19.
> WOOD PRODUCTS. By T. W. JONES. Pp. 19.
> THE HEAVY CHEMICAL INDUSTRY. By REX FURNESS. Pp. 28.
> FINE CHEMICALS. By T. W. JONES. Pp. 20.
> THE FERMENTATION INDUSTRIES. By REX FURNESS. Pp. 19.
> CHEMISTRY IN RELATION TO FOOD. By G. W. MONIER-WILLIAMS.
> Pp. 20.
> CHEMISTRY IN THE MANUFACTURE OF PIGMENTS, PAINTS AND VARNISHES.
> By C. A. KLEIN. Pp. 24.

London: Ernest Benn, Ltd. 1924.   Price 6d. each net.