JOURNAL
OF THE
SCIENCE OF FOOD AND AGRICULTURE

Published by the Society of Chemical Industry

Volume 6 | No. 12 | December, 1955

Journal of Applied Chemistry

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JOURNAL

OF THE

SCIENCE OF FOOD AND AGRICULTURE

1955, VOL. 6

Published by the Society of Chemical Industry
56 Victoria St., London, S.W.1

AUTHOR INDEX, 1955

SUBJECT INDEX, 1955

Printed in Great Britain for the Publishers by Butler & Tanner Ltd., Frome and London

MARSH SPOT OF PEAS: A REVIEW OF PRESENT KNOWLEDGE

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The history of the disorder of peas known as Marsh Spot is reviewed and its relation to manganese deficiency in the plant caused by deficiency or unavailability in the soil is discussed. The effect on the seed may be prevented by spraying a solution of a manganese salt on the crop at flowering time, but several applications at intervals during growth are probably necessary to ensure a healthy crop. Other advantages of the use of manganese are also mentioned and reference is made to the effect of Marsh Spot on consumer quality.

Introduction

IT is now established that Marsh Spot is due to lack of manganese in the pea plant, and that various disorders in other crops are induced by deficiency of this element. There are twelve elements essential for normal healthy plant growth. Some of these, like phosphorus and potassium, are required in relatively large quantities and are therefore known as the major elements; others like boron, iron—and manganese—are needed in very small amounts, so are termed trace elements.

Manganese performs important functions in the plant. It is closely associated with iron in the formation of chlorophyll, and also acts as a catalyst, aiding various oxidation and reduction processes within the plant. Thus the dangers of deficiency will be readily appreciated.

Historical

The characteristic brown spots found in the centre of pea seeds, for which the name Marsh Spot has been given, were first reported in Britain in the *Gardeners' Chronicle* during 1845, when an article entitled 'New Disease in Peas' appeared,[1] describing peas 'curiously affected with rot, yet so fair in appearance that a dealer in Mark Lane would have bought them without suspicion'. That the disorder was known much earlier was brought out in a letter published in the same journal[2] shortly afterwards in which the writer stated that the supposed 'new disease' was considerably older than the previous correspondent's great-grandfather.

It first appears to have been recorded in Holland by Mansholt in 1894,[3] where it is variably known as 'Bad Hearts', 'Bad Cores', 'Black Cores' and 'Black Kernels'. The same seed symptoms have also been described in Germany ('Bad Hearts') by Kirchner;[4] in the United States by Wade & Zaumeyer[5] and (in beans) by Orton & Henry;[6] in Finland by Jamalainen;[7] in Eire by Walsh & Cullinan;[8] and in Australia ('Hollow Hearts') by Myers.[9] The disorder is also known in France ('Maucheté').

It is interesting to note that Marsh Spot was not definitely attributed to manganese deficiency until the late 1930's following upon the work of Ovinge,[10, 11, 12] Löhnis[13] and Koopman[14] in Holland, and Pethybridge,[15] Heintze[16] and Lewis[17] in this country, although manganese was confirmed long previously as an essential element for plant growth.[18]

As long ago as 1917 Cayley[19] associated symptoms in affected peas with bacterial infection. This was disputed later by de Bruijn[20] and Lacey,[21] who were unable to isolate any micro-organism that could be responsible for the condition; they suggested that Cayley seemed to have been investigating a condition caused by two 'diseases' simultaneously. Van der Lek[22] pointed out that Marsh Spot developed in seeds as they matured, and postulated that as they dried out at harvest, so the substratum became unsuited for the (assumed) organism responsible for the spot, so that it died off. This was advanced as a theory as to why no causal organism had been found, and it was suggested that, as the disorder did not seem to be seed-transmitted, this pointed to the fact that the organism concerned was parasitic. The same author advanced other theories, suggesting infection of the seed via stigma or style or, under wet conditions, through water entering the seed near the embryo and setting up a rot. It was also suggested that the trouble might be physiological in character.

The idea that Marsh Spot was heritable persisted until the early 1930's,[23] but following five years' research, de Bruijn[20] disputed the suggestion that it was caused by an organism or virus and concluded it was a physiological condition. This was supported by British opinion[24] that it was related to soil type, and also that the necrosis and the fact that every seed and every plant was not necessarily affected were all characteristic of a physiological disorder. In America Orton & Henry[6] reported that the condition they described affecting beans seemed heritable, but perhaps this was a case where affected seed was planted again on deficient land. De Bruijn went further, suggesting that manganese or boron might be the key to the problem. She recommended soil improvement and hastening maturity (accomplished in experiments by unbalanced potassic manuring) as ways of combating the disorder, since protracted ripening conditions apparently favoured its development.

Previous work on boron deficiency of beet (' Heart Rot ') led Löhnis[13] to suggest that lack of this element was the cause of Marsh Spot, but analysis of healthy and diseased peas showed no relationship. This confirmed the findings of Ovinge[10] who noted that Marsh Spot was most prevalent in Holland in the young polders not long since under sea-water which has a high boron content. He concluded that boron was not the cause, but suggested that as Marsh Spot increased in degree with increasing alkalinity, manganese deficiency might be the cause, because it was already known to be a problem with other crops on alkaline soils.

Using a colorimetric method, Löhnis produced evidence that affected peas had a lower manganese content than healthy ones. This was confirmed by Glasscock & Wain[25] who agreed that the lower content of diseased peas might be due partly to the migration of the cell contents from the necrotic tissue. Heintze[16, 26] and Walsh & Cullinan[8] found the differences in manganese content small and insignificant.

Deficiency of manganese in the plant and seed was thus strongly suspected as the fundamental cause of Marsh Spot, other possible reasons such as contact of the pods with the soil, presence of common salt, phosphate and potash deficiency, and the system of cropping, all being discounted.[27] In many cases, however, the soils were found to contain quite adequate supplies of manganese, and eventually it became apparent that deficiency symptoms were due to unavailability of the element through the inter-relation of various factors in the soil including soil composition and reaction, and biological activity.

For information, the most recent summary of knowledge on Marsh Spot is by Lammers[28] of Holland.

Symptoms of deficiency and damage done

Effect on plant growth

Depending on the degree of deficiency, pea plants may or may not display visual symptoms of manganese deficiency. If there is an effect, this generally takes the form of intervenal chlorotic areas on the leaves, beginning at the leaf margins,[29] a feature being that deficiency usually occurs in patches. Sometimes growth may be retarded, the leaves being small with occasional brown necrotic spots. Those few pods which do develop are poorly filled.[30] In the presence of acute deficiency, growth is normal until manganese in the seed is used up ; then the young tendrils and internodes turn brown, the young leaves develop small discoloured areas of collapsed tissue between the veins and the older leaves assume a mottled yellow appearance, the veins showing as a green network. New leaves are even worse affected and eventually there is death at the growing point before flowering and podding.[18, 31, 32]

Effect on the plant's seed

The brown spot in the resulting mature seed is the most well-known and most characteristic symptom of manganese deficiency in peas (see Fig. 1). It indicates partial deficiency, because acute deficiency leads to premature death of the plant before pod-setting. The effect on the seed has been well described by de Bruijn[20] and Pethybridge.[24] The main bulk of the pea seed comprises food (endosperm) for the early stages of growth of the young plant, which is stored in the two cotyledons. Manganese deficiency in the seed is usually identified by a discoloured spot in

the centre of the adaxial surface of each endospermous cotyledon, readily seen if the seed is split open after softening by soaking. There is generally a small black spot in the middle, with a brownish-red discoloration round this, and the whole may be surrounded by further discoloration in the form of a dark halo. Microscopically, the discoloration is seen to be due to the death of cells below the skin of the cotyledons. The starch grains (part of the food material) in these cells are replaced by a yellowish-brown substance which eventually discolours the cell walls and also invades the intercellular spaces. Usually a plant responds to such conditions by forming a layer of cork cells to cut off damaged tissue from healthy tissue, but this does not occur with Marsh Spot, probably because the necrosis occurs in the ripening period when the cells have reached a resting stage. Sometimes the dead cells shrink and a cavity forms below the cotyledon skin. The skin may split, or if it remains attached to the dead tissue, a pit results.

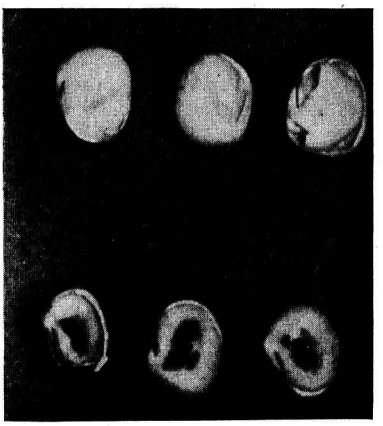

FIG. 1.—Top: *Healthy seeds cut open*
Bottom: *Seeds affected with Marsh Spot*

The size of the spot varies. It can be quite small, and, at the other extreme, it can extend to the outside of the seed where the discoloration should not be confused with *Ascochyta* spp. The plumule may be affected where the discoloration is extensive but it is rare to find the damage confined solely to the plumule. Extensive cotyledon necrosis predisposes the seed to attack by secondary bacterial or fungal organisms which may aggravate the damage. The lesions never extend over the whole inner faces of the cotyledons,[31] while it is reported from Finland[7] that affected peas may be identified externally by their wrinkled appearance. Normally, however, there are no external symptoms affecting the seed. Sometimes there may also be no visual internal discoloration, but merely denseness of the centre tissue.[24]

All the pods on a plant or all the peas in a single pod are not necessarily affected, and well-filled pods and large seeds are more affected than lesser-developed pods and small seeds.[33] This corresponds with the greater degree of infection found at the later stages of ripening,[13, 20, 21] and which can probably be ascribed to greater manganese availability for earlier-formed seeds.

Effect on germination of affected seed

Germination of affected seeds can be impaired. De Bruijn[20] reported losses when the proportion of damaged peas in a sample reached 20%. Doyer[34] states that work at the Dutch Seed Testing Station shows that if both cotyledons are seriously affected, together with the plumule tip, the seed usually fails to germinate, and this is confirmed by an earlier publication of the Official Seed Testing Station, Cambridge.[35]

The degree of infection varies from year to year, and generally has an insignificant effect on germination capacity. Pethybridge[24] states that seedlings grown from affected seed may be normal, slightly undersized, or—more commonly—defective, this occurring mostly where the cotyledons and the plumule are damaged (see Fig. 2). The leaf may be completely suppressed or reduced to a stub with ragged stipules. The leaflets may be puckered with wrinkled margins, and develop a 'hooded' appearance. If the plumule is affected, the terminal bud is often killed and two, usually unequal, shoots develop in the axils of the cotyledons. These lateral shoots generally allow the plant to make good, provided soil and weather conditions promote early development, and conditions continue to favour growth.[22]

The Dutch seed certification authority (N.A.K.) permits not more than 25% infection, but only 10% if the plumule is damaged.

Distribution

Marsh Spot is likely to occur in many areas because manganese-deficient soils are widespread. Wallace[29] states that deficiency is particularly prevalent in the Fens, Romney Marsh and Yorkshire, and occurs sporadically in Somerset, Wiltshire, Shropshire, Staffordshire and North Wales, usually arising where the following soil conditions obtain :

(1) Thin peaty fen soils overlying calcareous sub-soils (' skirt ').
(2) Alluvium and marsh soils derived from calcareous materials, such as calcareous silts and clays or shelly marine sands and muds.
(3) Poorly-drained calcareous soils with high organic matter content, e.g., wet areas overlying the chalk or carboniferous limestone formations.
(4) Calcareous black sands and reclaimed acid heath soils subsequently limed too generously.
(5) Calcareous soils broken up from old grassland.
(6) Old black garden soils where stable manure and dung have been applied for many years.

FIG. 2.—*Effect on seedlings grown from seed affected with Marsh Spot, after 15 days in sand*

Left : *Tip of plumule affected and formation of lateral leaf*

Right : *Growing point of plumule dead, and shoots from buds (normally dormant) in axils of cotyledons*

Following is a summary of the position in the different Provinces of the National Agricultural Advisory Service.[35a] To supplement this information it may be noted that it has been recorded in Lincolnshire, Cambridgeshire, Kent, Devon and Denbigh in the past fifteen years.[36, 37]

Northern Province

There are no well-defined areas that suffer from manganese deficiency, but isolated cases are reported every year—chiefly on oats (Grey Speck). The area immediately south of Berwick (carboniferous limestone) and the magnesian limestone area of East Durham are most affected. In the Berwick region there are post-glacial lakes with peaty material over a marl of fresh-water snail shell remains (calcareous material). Peas are an unimportant crop.

Yorks. and Lancs. Province

Although a large acreage of peas is grown in Holderness (boulder clay) and on the south side of the Ouse in the Goole area (warp land), no cases of Marsh Spot have been recorded in recent years. Deficiency in other crops, like oats, is not uncommon in various areas.

East Midland Province

Incidence of visual symptoms of manganese deficiency in peas is slight (two cases) but it is almost certain that seed symptoms have developed in other pea crops. Deficiency occurs with other crops.

West Midland Province

Manganese deficiency is a problem in the Carrington district of Cheshire (Mersey Valley) where green peas are becoming more important. Marsh Spot has not yet been recorded.

Eastern Province

Marsh Spot is fairly common, mostly on alkaline soils such as originally poorly-drained pasture land with a high water-table. Fenland peat and silt soils are affected where the manganese content is high but rendered unavailable by biological activity. On acid heath soils (e.g., in Norfolk), overliming can lower the soil manganese content and cause deficiency.

Eastern Province Sub-centre, Kirton

Incidence of Marsh Spot in Holland (Lincs.) is not great. It occurs in the Gosberton area (organic loamy fine sand with acid reaction, originally dunes) and at Crowland on ' skirt ' soil (organic fine sandy acid loam overlying river gravel). It is also said to occur in Holbeach Marsh where there are a few old sand-dunes similar to those at Gosberton.

South-eastern Province

Deficiency is chiefly found in the Romney Marsh district of Kent and East Sussex where a large acreage of peas is grown. Marsh Spot occurs quite frequently and control measures are practised.

South-western Province

Oats, sugar beet, etc., sometimes suffer from manganese deficiency, but Marsh Spot has not been recorded for some years, probably because few peas are grown in the region. Deficiency has been recorded on granite soils in Cornwall as well as around Helston and Padstow (shales) due to overliming; also near Bridgwater, Somerset (lower lias), and north-east of Bristol (Severn alluvium) where the soils have appreciable organic matter and tend to be alkaline.

Welsh Province

Marsh Spot has not been recorded in recent years. Oats are spasmodically affected in some years, chiefly in Flintshire on neutral or alkaline soils derived from carboniferous limestone.

Welsh Sub-centre, Cardiff

Manganese deficiency in oats and barley has been recorded on the alkaline lias clay soils in the Penarth–Barry area of Glamorgan; also on the coal measures on overlimed peaty soils.

Welsh Sub-centre, Bangor

Marsh Spot is rare as peas are not grown extensively. Deficiency in oats occurs sometimes in areas where peaty soils overlie calcareous material; also on alluvial and marsh soils, and occasionally on reclaimed heath.

Conditions inducing deficiency

The following conditions normally exist wherever manganese-deficient crops are encountered[29] and, in combination, these seem to immobilize the soil manganese: (1) high organic matter content; (2) poor drainage; (3) high water-table; (4) high pH (alkalinity).

Furneaux & Glasscock[27] found that the incidence of Marsh Spot was correlated with a high water-table, severe damage being noted only where the water-table was within 52 in. of soil surface. They disproved that wet weather was the cause and found that soil texture modified the effect of high water-table on Romney Marsh, heavier soils being the worse affected. Heintze[16] examined the soils covered by Furneaux & Glasscock's work, and found a closer relationship with soil reaction (pH) than with soil type or texture, Marsh Spot being absent on acid soils.

The effect of soil reaction has been elaborated on by Truog[38] who expressed the view that under acid conditions manganese exists in a divalent (reduced or manganous) form, behaving as an exchangeable cation like calcium and magnesium, being readily brought into solution in the soil as a bicarbonate through the ever-present carbonic acid. As the pH rises above 7, the divalent manganese is oxidized to the tetravalent form (probably manganese dioxide) by oxygen in the soil solution. As this tetravalent manganese is insoluble in carbonic acid, Truog claims

that it is not available to plants. He states that a slight acid reaction (pH 6·5) is the optimum for the availability of all plant nutrients. Soil-manganese increases in solubility (and availability) with increasing acidity and in many soils becomes largely unavailable above pH 6·5. This is borne out by Ovinge's results quoted by Koopman :[14]

pH	Marsh Spot %
7·4 (alkaline)	6
6·7 (slightly acid)	4
6·2 (acid)	0
5·6 (very acid)	0

Heintze & Mann[39] put forward the hypothesis that part of the manganese of neutral and alkaline organic soils is rendered unavailable due to retention by the organic matter of divalent (available) manganese formed by reduction. This view is supported by results of later experiments carried out by the same investigators.[40]

Earlier work by Heintze[26] indicated that deficiency is not always related to the *total* manganese content of the soil. Fractions defined by extraction methods as 'exchangeable' manganese (manganous ions held by the clay and organic colloids, but capable of being displaced by other ions, e.g., calcium through liming) and 'readily reducible' manganese (oxides of manganese in the soil capable of easy reduction) were not found to be accurate guides in characterizing manganese-deficient soils. Leeper,[41] on the other hand, found the 'readily reducible' manganese fraction helpful in identifying deficiency on neutral and alkaline soils. In reviewing the factors affecting availability, the same worker[42] enlarges on the 'readily reducible' theory, and in other papers Jones & Leeper[43, 44] discuss how these reducible oxides can cure manganese deficiency in peas.

Quastel *et al.*[45] have drawn attention to the oxidation/reduction processes which influence the availability of manganese through the activity of soil micro-organisms. They described the soil-manganese status as being the result of a dynamic equilibrium between the di-, tri-, and tetravalent forms of manganese compounds in which biological activity plays a role. They suggested that increasing the number of manganous ions by encouraging the reduction processes might be an important step in preventing manganese deficiency, and this helps to explain why Marsh Spot does not occur on waterlogged soils, i.e., where the anaerobic conditions favour biological reduction. This statement appears to contradict the fact that Marsh Spot on some marsh soils has been associated with a high water-table,[27] but Heintze[26] explains that the ground water in such soils is highly alkaline and tends to cancel out the effects of reduction.

Lammers[28] refers to another cause of manganese 'fixation'. This occurs on clay soils of low lime status where the manganese is adsorbed by the clay complex. Here liming appears to relieve the deficiency, the calcium replacing the adsorbed manganese.

The incidence of Marsh Spot also appears to be associated with inorganic nitrogen in the soil[26] and the disorder has been aggravated by applications of nitrogenous fertilizers. Heintze[26] postulated that Marsh Spot occurred commonly on ploughed-up grassland due to liberation of much inorganic nitrogen. Another theory referred to by Lammers[28] is that the large reserves of organic matter on ploughed-up grassland stimulate micro-organisms which oxidize the manganese to an unavailable form. It seems that manganese is connected with the nitrogen metabolism of the plant, because Marsh Spot can also be induced by injecting nitrogenous compounds into the plant itself.[26]

Manganese toxicity

By contrast, in strongly-acid soils manganese may be present in toxic concentrations, causing crop failures ascribed to soil acidity.[29] It might also arise occasionally through excessively heavy dressings of a manganous salt applied for Marsh Spot control, the margin between sufficiency and excess being far narrower than for the major essential elements. This is unlikely to occur, however, since manganese-deficient soils usually 'fix' any manganese applied. Toxicity may be corrected by liming and loose cultivation to aerate the soil, so encouraging the oxidation processes which reduce availability.

Cory[46] tabulates the position as follows:

Reaction	pH value	Incidence of manganese disorders
Alkaline	8	Deficiency may occur at these levels, especially on organic soils
Neutral	7	
Slightly acid	6·5	
Markedly acid	6	Deficiency unlikely
	5	
	4	Risk of toxicity increases below pH 5

Correction of deficiency

The control of the disorder may be considered under three headings as follows:

Application of available manganese to crops

Pethybridge[15] reduced the number of affected seeds in a crop by applying manganous sulphate in water to the soil in mid-June, repeating the spraying a week later. Koopman[14] also obtained good results from spraying with manganous sulphate, the percentage of Marsh Spot incidence being reduced from 11 to 0·5 in the variety *Zelka*. In the same year Ovinge,[11] using the salt in powder form, found application to marrowfat peas at flowering time with about 1 cwt. per acre resulted in 7·6% affected seeds compared with 25·8% for untreated peas; 2 cwt. per acre reduced the damage to 1·6%. In four other experiments designed to test early application, treatment at flowering with 1 cwt. per acre was far better than treatment when the peas were 4 in. in height. These findings were confirmed by the same investigator[12] in 1937. Spraying before flowering with 0·1% manganous sulphate reduced attack by only 50%, but three sprayings (two after flowering) gave extremely good control.

Further work was carried out in this country on the Romney Marsh by Lewis.[17] Heavy dressings of a soluble manganous salt in powder form applied at sowing time had little effect. Later soil applications at flowering time were more effective but spraying at this time gave even better results. Spraying at flowering was more effective than before or after flowering, and the following tentative recommendations were made by Lewis for the benefit of farmers:

Per acre at each spraying

Spraying once when in flower

24 lb. of anhydrous manganous sulphate or 36 lb. of hydrated manganous sulphate in 100 gal. of water, plus wetting agent.

Spraying twice when in flower

12 lb. of $MnSO_4$ or 18 lb. of hydrated $MnSO_4,4H_2O$ in 100 gal. of water, plus wetting agent.

The purpose of two applications is to overcome the effect of rain following the first application. Powder application to the soil is inferior to spraying because the results are unpredictable and the manganese applied is easily fixed or converted to an unavailable form.[47] Soil application is dependent on rain to bring it into solution for the plant. Spray application at flowering allows the available manganous manganese to be absorbed directly into the plant via the foliage.

These findings are supported by Heintze[26] who found that soil application of manganese as manganous sulphate and basic slag gave only partial control of Marsh Spot. Other methods of administering manganese corroborated the view that late sprayings were the most effective; no Marsh Spot occurred where plants were injected with manganous sulphate at the flowering stage, but treatment before the bud stage resulted in 21% infection. Soaking the seed in manganous sulphate solution prior to sowing also failed to correct the disorder, confirming that it is *late* availability which is the crux of the problem. Manganese accumulated before flowering is ineffective in preventing Marsh Spot; the disorder is controlled only if adequate manganese is supplied throughout the seed formation period.

Differences in susceptibility between varieties

Quanjer[48] noted that several kinds of peas were susceptible to Marsh Spot including grey and green peas. De Bruijn[20] listed varieties noted in the Reports of the Dutch Government Seed Testing Station as being affected, including brown and fresh runner-beans. Lacey[21] observed that late or main crop varieties were damaged more than earlies. That the heaviest peas of a sample are worse affected, particularly in lesser-damaged samples, was noted by de Bruijn[20] and confirmed by Furneaux & Glasscock.[27] De Bruijn also concluded from fertilizer experiments that a long ripening period encourages Marsh Spot.

In their experiments Koopman[14] and Ovinge[11] found that the large-seeded marrowfat variety *Jumboka* was more heavily attacked than the variety *Zelka* with smaller seeds. This confirmed the views of de Bruijn.[20] Assessments reported by Koopman showed that sprayed *Jumboka* peas produced seeds with 6% incidence of Marsh Spot whilst the figure for unsprayed peas was 27·75%. The corresponding values for *Zelka* were 0·75% (sprayed), and 8% (unsprayed).

Glasscock[49] found that the varietal characters of large seed size and late season favour the disorder, even within a single variety. Plot trials with large-, medium- and small-seeded, round and wrinkled, and early, second early and late varieties gave results which agreed well with information obtained from a seed firm, and showed the following varietal characters to discourage the incidence of Marsh Spot: (1) early maturity; (2) small seed; (3) round seed (associated with early maturity).

Improving the availability of soil manganese

Heintze[26] has shown that crops sensitive to manganese deficiency are usually healthy on soils with more than 0·3 mg.-% of exchangeable manganese, and sometimes with less. Recently Quastel *et al.*[45] have put forward various methods which might be developed for rendering soil manganese more readily available to the plant, as follows:

1. Reduction of soil reaction (pH) until the kinetics of the manganese 'cycle' give an increase of di- over tri-valent manganese.
2. Inhibition of oxidizing reaction in the 'cycle' by specific poisons such as sodium azide.
3. Stimulation of reducing agents by suitable manuring and certain cultural operations.
4. Stimulation of manganese-reducing organisms relative to oxidizing organisms.

High pH is well known as being associated with Marsh Spot and the danger of overliming is generally appreciated in this respect. Liming also increases aeration which favours manganese unavailability.

Sulphur has been used to increase manganese availability though its effect is uncertain.[26] Formerly it was thought that its action was due to a lowering of the pH by the supposed formation of sulphuric acid by oxidation, probably by bacteria. However, more recently it has been shown that sulphur can increase divalent manganese in the soil before a change in pH occurs, and this may be ascribed to the formation of thiosulphates, intermediate oxidation products, which behave similarly but more rapidly, without changing the pH. Thiosulphates are non-acid and act as reducing agents. Broadcast thiosulphate can severely damage peas but placement of sulphur and sulphur-containing compounds may have some application in the future, subject to sulphur becoming less costly.[45]

Marsh Spot control in practice

In practice, application of manganese to correct deficiency in peas is carried out to a limited extent, probably due to lack of symptoms during growth. In certain fenland and marsh areas, e.g., Romney Marsh, Kent, where the disorder is more widely recognized, treatment is often regarded as an essential routine measure. Although the chloride, citrate or sulphate of manganese can be used, the last is generally chosen because of its cheapness. Most growers who treat their crops realize that it is important to spray at flowering time to prevent the appearance of Marsh Spot in the seeds and normally use 5–20 lb. per acre of manganous sulphate (together with a spreader to assist the spray to adhere to the leaves) applied by means of a high-volume

spraying machine. At about 75*s*. per cwt. for the salt, the cost per acre for material need not exceed 15*s*.

In the experience of some farmers, two or three small dosages of 1–2 lb. per acre at intervals up to and including flowering time are more effective than a single higher dosage at the time of flowering. It is considered that the early applications correct the deficiency in the young plant, producing uniform healthy vigorous growth, whereas treatment at flowering time has the effect of preventing the symptoms in the resulting seed. In this connexion some growers mix their manganous sulphate with DDT for controlling pea weevil in the early stages of growth, and again at flowering time to combat greenfly or pea moth maggot. Manganous sulphate has also been mixed and applied with dinoseb for eradicating weeds in the crop. Whenever mixtures are applied, however, it is most important that a pure manganese salt be used.

MCPA liquid weedkillers are not recommended for use in peas, but it may be mentioned, in relation to other crops which suffer from manganese deficiency, that the mixing of manganese salts with MCPA is not advisable, especially if low-volume application is contemplated ; similarly a DDT emulsion is likely to prove less compatible than wettable powder formulations. Admixtures or use of impure materials may cause nozzle blockages, though this may sometimes be overcome in practice by removing the nozzle filters.

The main criticism against spraying to control Marsh Spot is the mechanical damage caused to the crop, since treatment must be carried out at flowering time for best results. Such damage may be partially prevented by using a wide-boomed spraying machine, parting the rows by hand in advance to correspond with the wheels of the tractor and sprayer. Alternatively, the rows may be parted by dividers fitted on the tractor. Whatever the disadvantage of spraying at flowering time, the fact remains that it has been shown to be the only really effective method of control.

The other important factor growers should bear in mind is to apply lime with extreme caution, especially on black fen and ' skirt ' (neutral or slightly acid) soils. The shell marl form of black peat is particularly susceptible to Marsh Spot.

Other effects of applying manganese to peas

Influence on yield

In experiments carried out by Koopman,[14] besides controlling Marsh Spot by means of manganous sulphate spraying, it was found that the 1000-seed weight of treated plots was higher than untreated. The value for sprayed *Jumboka* peas was 455 g. and unsprayed 417 g. The corresponding figures for the variety *Zelka* were 350 g. and 345 g. This indicated an increase in yield due to treatment and it seems that this can occur on some occasions.

Lewis[17] obtained up to 81% extra yield of pods in one experiment, all manganese treatments increasing yields except one heavy soil application. Roach[50] obtained a yield increase of 11 cwt. per acre of peas (unsprayed 9 cwt. ; sprayed 20 cwt.) which improved the value of the crop by £26 per acre for an outlay of £1.

It has also been reported[51] that peas affected with Marsh Spot tend to split more readily on threshing. This could indirectly contribute to a loss in yield.

Development of seeds

Since manganese deficiency may prevent the formation and swelling of some of the seeds, the addition of manganese can serve to fill-out undeveloped peas.

Effect on rate of maturation

Application of a manganous salt may delay ripening. Ovinge[11] reported that 2 cwt. per acre of manganous sulphate applied to the soil at flowering time gave the plots a fresher-green colour and caused them to mature ten days after the untreated plots.

Effect on colour of seeds

Lammers[28] refers to the fact that peas from the Groningen region of Holland often have an attractive dark-green colour compared with those from the western clay soils, which contain more

lime. In Groningen the soils contain little lime and it may be conjectured that manganese is more available, this being reflected in better seed colour as well as in foliage colour as already mentioned.

Effect on consumption quality

Marsh Spot is not of importance with vining peas for canning or quick freezing because it does not develop until the later stages of maturity. Investigations by Veenbaas[52] in Holland revealed that with dry harvested peas, up to 15% infection has very little effect on consumption quality, but when the figure reaches the order of 30%, taste is very unsatisfactory. Affected portions of seeds possess a tougher texture and cook unevenly. Seeds affected externally are the most serious in influencing quality, such peas readily bleaching. Storage of affected samples for 3 to 5 months in the winter in a dry, cool dark place caused no significant change either in the proportion of peas affected or the degree of damage, and did not alter the consumption quality.

By statute, the maximum permitted infection in consumption peas in Holland is 15%, of which not more than 4% may be seriously affected.

Acknowledgments

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STARCH AND VANILLIN

By R. RUTGERS

Vanillin, when added to maize or potato starch in concentrations of 0·1 to 1·5%, becomes bound to the starch in the course of a few weeks so that it is no longer removed completely by ether extraction. Almost complete recovery can, however, be obtained by gelatinization of the starch in cold aqueous alkali and ether extraction after acidification.

Waxy maize starch showed much less extensive and rapid binding of vanillin. As a possible explanation of the difference between the two types of starch, the packing of vanillin in the amylose spirals is postulated and discussed.

Introduction

An estimation of vanillin in an old sample of custard powder (maize starch), known to contain originally about 100 mg. per 100 g., and which had been kept for some months in a paper sack, gave only 10 mg. of vanillin per 100 g. The method of analysis consisted of a fourfold extraction with ether, followed by evaporation of the solvent, dissolving the residue in neutralized ethanol, and titrating with 0·1N-sodium hydroxide. In a freshly prepared sample of custard powder there was found about 90% of the added quantity of vanillin using the same method of determination.

Extraction of the old sample with chloroform gave even lower results.

The question arose whether the vanillin could have evaporated from the sample, or if it could have decomposed, or have been bound, e.g., in ester-form. The starch was therefore gelatinized in the cold with sodium hydroxide, and after acidifying with hydrochloric acid, an extraction with ether was performed. In the washed extract there could be titrated an amount of acidic material, equivalent to 75 mg. of vanillin per 100 g. of starch. The extracted substance had the odour of vanillin (with off-odours), and gave positive reactions for phenol and aldehyde groups with ferric chloride and with phloroglucinol and hydrochloric acid, respectively. The same reactions were negative or very faint with the direct ether extract of the old sample and positive with the ether extract of a freshly mixed sample of maize starch and 0·1% vanillin.

The vanillin did not appear to have evaporated or decomposed, at least not for the major part. It seemed to have become bound to the starch, sufficiently strongly not to be extracted with ether or chloroform and yet sufficiently loosely to be liberated in a cold aqueous alkaline solution. Indeed old samples of starch–vanillin mixtures showed a considerably more feeble vanillin odour than did fresh samples.

It seemed of interest to investigate the binding of vanillin in the course of time for different kinds of starch and for different concentrations of vanillin.

Experimental

Methods of analysis

Extraction of powder with ether.—Technical ether, dried over sodium sulphate, was used. A weighed amount of 10–20 g. of starch–vanillin mixture was shaken at room temperature with 35 c.c. of ether and centrifuged. The procedure was repeated 4 or up to 10 times. The ether was distilled off almost completely from the combined extracts and the residue dissolved in 50 c.c. of neutralized ethanol and titrated with 0·1N-NaOH using phenolphthalein as indicator. (By titrating in alcoholic solution pure vanillin gives practically 100%.) Further, an excess of 5 c.c. 0·1N-NaOH was added to the solution, the mixture boiled for 4 minutes and back-titrated with acid after cooling. The ' ester ' so found was calculated also as vanillin (equivalent weight 152).

Extraction after cold alkaline gelatinization.—To 20 g. starch–vanillin mixture suspended in 200 c.c. of distilled water, 17 c.c. of 30% (w/w) sodium hydroxide solution was added with stirring. After keeping at room temperature for 30 min., the solution was acidified with 40 c.c. of conc. hydrochloric acid (38%). After 30 min., the mixture was diluted to about 600 c.c. and extracted

four times with 100 c.c. of ether. For ease of separation of the layers, some alcohol can be added. The combined ether extract was washed about 5 times with water, until the acidity of the wash water was equivalent to less than one drop of 0·1N-sodium hydroxide. After drying over Na_2SO_4, the procedure of evaporating and titration was followed as stated above.

With both methods blank determinations on starch alone were performed.

The accuracy of the titration is about 5 mg. of vanillin per 100 g. of mixture.

Materials

Starches:	Maize starch	ordinary trade samples
	Potato starch	ordinary trade samples
	Waxy maize starch	'Amioca' from Stein-Hall, New York
Vanillin:	Pure grade, 100%, relatively coarse powder	

The mixtures of starch and vanillin were prepared by dry-mixing for some minutes with a spoon. The samples were kept in brown glass-stoppered bottles in a dark room at room temperature. The mixtures were analysed directly and after 1 week, 6–7 weeks and 3–3½ months.

Blank determinations

The maize starches gave a blank value of about 15 mg. (expressed as vanillin) per 100 g., by direct fourfold ether extraction, and 5 mg. of 'ester'. By the gelatinization method we found approx. 25 mg. 'vanillin' plus 15 mg. of 'ester'. For potato starch, the direct titration gave lower figures, 10 and 15 mg., respectively, for direct extraction and extraction after gelatinization, and practically no ester values.

The estimations in starch–vanillin mixtures were corrected for the blank values. For the mixtures the 'ester' values had increased insignificantly for both maize starches, by both methods of determination, with an average of about 5 mg. per 100 g. Occasionally we found a few values of 20–40 mg. For potato starch somewhat increased ester figures were obtained, about 10–15 mg. on an average. In any case we found by direct ether extraction no ester values approaching the amount of added vanillin (100–1500 mg. per 100 g. of mixture), neither for fresh nor for old samples. In the following we give only the direct titration figures, no ester values.

Results

In Table I are given the results expressed in mg. per 100 g. of experiments with 0·1–0·15% added vanillin using both methods of analysis. For the estimations of vanillin, 20 g. of mixture were used, with fourfold extraction for the direct ether method.

In Fig. 1 are given the results of keeping tests with higher vanillin concentrations (1·5%). In these cases only direct ether extractions were made with 10 g. of sample and 5- or 10-fold extraction. The results are expressed in % of the added vanillin.

Discussion

The figures for vanillin, estimated by the gelatinization procedure, varied somewhat erratically. It may be that the method needs further precision and more prolonged ether extraction. The figures however showed no systematic decrease with longer keeping-time.

For the direct extraction method and low vanillin concentrations we can observe a steep decrease in the amounts of extracted vanillin when the samples are stored, both for maize starch and potato starch. After 2–3 months, direct ether extraction gave very low results. As a method of analysis the extraction with ether (or chloroform) is totally unsuited for old samples of custard or pudding powders containing vanillin. Even a 10-fold extraction with ether gave very low results, as the figures in the graph show. Somewhat higher figures were obtained as the number of extractions was increased. This indicates that vanillin is not easily extracted with cold ether. Probably the determinations in fresh samples with lower vanillin concentrations (Table I) would have been higher and nearer to the calculated contents, if more ether and more extractions had been used.

Table I

Vanillin (mg./100 g.) recovered from starch–vanillin mixtures after various times of storage

	Direct extraction method	Extraction after gelatinization
Maize starch		
100 mg. of vanillin per 100 g.		
Immediately after mixing	101	91
After 1 week	55	66
After 6 weeks	23	85
After 3½ months	11	83
Maize starch		
150 mg. of vanillin per 100 g.		
Immediately after mixing	117	101
After 1 week	37	88
After 7 weeks	12	80
After 3 months	15	106
Waxy maize starch		
150 mg. of vanillin per 100 g.		
Immediately after mixing	133	97
After 1 week	91	75
After 7 weeks	32	87
After 3 months	13	92
Potato starch		
150 mg. of vanillin per 100 g.		
Immediately after mixing	132	114
After 1 week	32	127
After 7 weeks	6	85
After 3 months	15	98

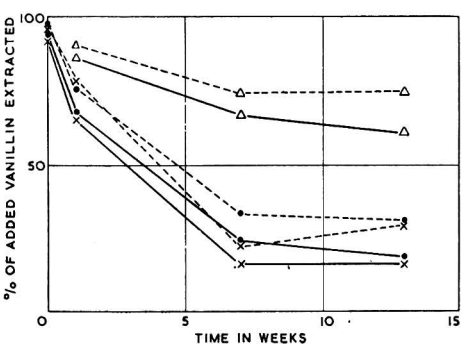

FIG. 1.—*Ether extraction of vanillin from starch mixtures (1500 mg. per 100 g.) after various times of storage*

5-fold extraction
10-fold extraction
● = maize
△ = waxy maize
× = potato

With waxy maize starch the decrease in the proportion of extracted vanillin was less steep for the samples with lower contents of vanillin (100–150 mg. per 100 g.), but after some months the same low figure as above was found. With higher vanillin concentration (1500 mg. per 100 g.) the difference between waxy maize and maize or potato starch was more pronounced. The latter starches showed a steep decrease to low figures (approx. 20–30% of the original values), while

waxy maize gave a much less pronounced decrease, and very appreciable figures (approx. 70%) for 3-month-old samples. The values for potato starch were a little lower even than for maize starch. The extractable vanillin seemed to reach constant values after about 2 months. The residual vanillin contents of Fig. 1 were higher than the 100–150 mg. added in the series shown in Table I.

For the samples with lower concentrations, the odour of vanillin was less pronounced in the sequence potato < maize < waxy maize, for 3 months' old samples, which had practically the same very low figures for ether-extractable vanillin.

Vanillin is bound slowly in starch, and rather less so in waxy maize starch, but is recoverable to a large extent after aqueous alkaline cold gelatinization of the starch. The vanillin is not bound as a soluble ester, as no significantly higher ester values were found on storage. It could be bound to the starch molecules as ether or ester or by ' adsorption '. This explanation is not disproved by the experiments. Perhaps however the increasing difference between 5- or 10-fold ether extraction for older samples compared with fresher samples (see Fig. 1) may be taken as some indication that the vanillin is not bound with chemical valencies. Furthermore the difference between waxy maize (rich in amylopectin) and maize or potato starch is puzzling, as maize and potato differ in many other respects. A possible explanation may lie in the fact that waxy maize starch has a very low amylose content, and one can postulate that vanillin is packed in the amylose spirals, as occurs with other substances.

We sought affirmation of this theory by investigation of the colour of the samples with iodine, and by observing the colour in ultra-violet light.

The Amioca starch used showed a red-purple colour with aqueous iodine solution and a light brown colour with iodine vapour at room temperature in an atmosphere saturated with water vapour.

With iodine, the 3 months' old samples containing 1500 mg. of vanillin per 100 g. showed practically no difference from the original starch, neither for waxy maize nor for maize starch. The amylose spirals have not become inaccessible to iodine molecules.

The colour of the samples was observed in ultra-violet light with a Philips Philora lamp (HPW-125W Typ. 57202E/70). Samples without vanillin showed a brownish colour for maize and waxy maize and somewhat more blue for the potato starch sample. With addition of vanillin (1·5%) freshly prepared samples showed more blue colours, more distinctly blue in the order Amioca > maize > potato; pure vanillin showed a dark blue purple colour. For 3 months' old samples there was little difference between Amioca and maize, but potato starch showed still a little darker blue colour. For all three starches the colour in ultra-violet light had become more blue on storage of the mixtures.

Inclusion compounds often show a more blue colour than the included substance itself. There was however no difference between old maize and waxy maize samples with vanillin. The generally more intense and darker blue colour of the old samples could be ascribed to the formation of inclusions, but could also be explained by a better distribution of the added vanillin by a process of evaporation and condensation. The evidence for inclusion formation is not conclusive.

The process of the binding of vanillin is a slow one at room temperature. If the vanillin is bound by packing in amylose spirals, or by other means, the vanillin can be thought to reach the binding places as vapour. The evaporation of vanillin is very slow. Very fine milling of the vanillin might accelerate the velocity of binding, as might also be the case if working at higher temperatures.

Acknowledgments

Thanks are due to Messrs. P. van Diepen and H. Rieke for their analytical help.

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Received 17 May, 1955

THE VOLATILE OIL OF *DACRYDIUM BIFORME*

By R. E. CORBETT and L. C-K. WONG

The essential oil of *Dacrydium biforme* has been fractionated in an efficient column and is shown to contain at least 15 constituents. Eight of these have been identified with known compounds, namely, myrcene, β-terpinene, (+)-longifolene, (+)-aromadendrene, (−)-metrosiderene, (+)-δ-cadinene, (+)-phyllocladene, and α-camphorene. Other constituents include oxygenated terpenes, and a solid sesquiterpene alcohol.

The genus *Dacrydium* which belongs to the pine family, *Podocarpaceae*, contains 16 species, seven of which are endemic to New Zealand. The volatile oils of two of these endemic species, namely *D. kirkii*[1] and *D. colensoi*,[2] have been examined in detail, while the oils of *D. cupressinum*[3] and *D. franklinii*[4] (native to Tasmania) have also been examined but not so completely. A preliminary investigation of the oil from *D. biforme*[5] has also been reported, and the sesquiterpene (+)-cadinene and the diterpene phyllocladene were identified. All the oils from these species contain phyllocladene and cadinene, and α-pinene is reported from all except *D. biforme*. The oil of *D. elatum*, which was examined by the Établissement A. Chiris in 1925,[6] differs markedly from other *Dacrydium* oils.

A complete examination of the spring oil of *D. biforme* has now been made. The presence of at least 15 constituents has been established, eight of which have been identified, including a new sesquiterpene.

Experimental

The oil (yield 0·34% v/w, 7–8 days after collection) collected in September had the following constants, d_{20}^{20} 0·9110, n_D^{20} 1·5018, $[\alpha]_D^{20}$ + 15·57°. It was separated in a preliminary fractionation into two portions, (*a*) terpenoid and sesquiterpenoid, and (*b*) diterpenoid. The lower-boiling fraction was then fractionated using a 90-cm. column of 48 theoretical plates after the pattern of Lecky & Ewell.[7] The first portion was distilled at 19 mm., and the remainder at 2·5–8 mm. The distillate was collected in 2·5-c.c. aliquots, and the progress of the fractionation was followed by plotting the boiling point, refractive index, density, and optical rotation as the distillation proceeded. These results are summarized in Table I, and in the refractive index/density diagram (Fig. 1) for the sesquiterpene fractions.

Table I

Fractionation of oil of D. biforme

Fraction No.	b.p., ° c/mm.	n_D^{20}	d_4^{20}	$[\alpha]_D^{20}$	% of total distillate	Product
1	58°/19	1·4707	0·7902	+ 0°	12·0	Myrcene
2	44°/8	1·4770	0·8401	+ 6°	0·5	β-Terpinene
3	three oxygenated terpenes				0·1	
4	80°/4	1·4908	0·9130	+ 15°	2·0	Tricyclic sesquiterpene
5	90°/4	1·5020	0·9300	+ 30°	8·0	(+)-Longifolene
6	94°/4	1·4992	0·9145	+ 21°	1·5	(+)-Aromadendrene
7	100°/4	1·5050	0·9148	− 2°	7·0	(−)-Metrosiderene
8	104°/4	1·5070	0·9185	+ 40°	2·0	(+)-γ-Cadinene
9	104°/4	1·5095	0·9168	+ 76°	7·5	(+)-δ-Cadinene
10	104°/2·5	1·4962	0·9550		0·5	Sesquiterpene alcohol
11			m.p. 136–137°		a trace	(?) Torreyol
12			m.p. 96°		35·0	Phyllocladene
13	160–170°/3·5	1·5223			0·1	α-Camphorene
Intermediate and minor fractions					8·5	
Residue and high-boiling fractions					15·3	

The very low density of fraction 1 indicates an acyclic terpene, and the physical constants are in close agreement with those reported for specially purified myrcene.[8] The identification of this fraction as myrcene was confirmed by the preparation of the characteristic adduct with maleic anhydride.

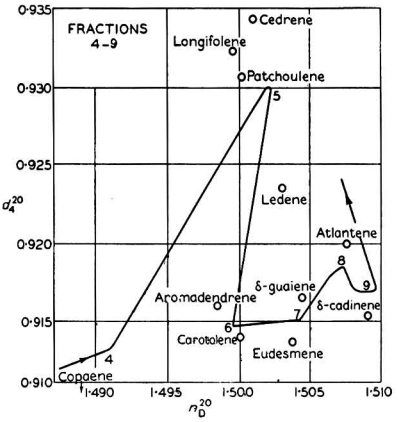

FIG. 1.—n/d *graph of successive fractions of distillate*

The physical constants of the small second fraction indicate a monocyclic terpene, and the presence of β-terpinene was established by the formation of the tetrabromide.[9] The very poor yield of this derivative and the optical rotation of the fraction indicate the presence of at least one other unidentified terpene.

A series of three very small oxygenated terpene fractions each less than the hold-up of the column, and consequently very impure, which separated the terpenes from the sesquiterpenes, were not examined. The physical constants and molecular refraction, 64·6, of fraction 4, the first sesquiterpene, indicate a tricyclic structure. These constants and the *n/d* plot are close to those of copaene and a tricyclic sesquiterpene isolated from the essential oil of *Phyllocladus trichomanoides*,[10] viz., b.p. 110–114°/10 mm., d_{25} 0·9189, n_D^{25} 1·4928, $[\alpha]_D$ + 20°. Further, these are the only sesquiterpenes so far reported, with boiling points as low as this fraction. Copaene yields cadinene dihydrochloride, while this sesquiterpene did not yield a crystalline hydrochloride. It is of the cadinene type since it yielded cadalene on dehydrogenation with selenium. Quantitative methylene and *iso*propylidene determinations[11] revealed the absence of such groupings. The infra-red spectrum showed a band at 1015 cm.$^{-1}$ which could be due to the presence of a *cyclo*propane ring in the molecule.[12] Thus, this sesquiterpene may be similar to copaene, possibly differing only in the position of the double bond. Permanganate oxidation under conditions employed by Briggs & Sutherland[10] for the preparation of copaene ketonic acid, yielded a ketonic acid, which gave an amorphous semicarbazone. Neither of these compounds could be obtained in sufficient purity for characterization. The small quantity of this fraction precluded further study of this tricyclic sesquiterpene, which is possibly identical with that reported by Briggs & Sutherland.[10] The tricyclic sesquiterpene reported in the essential oil of *D. colensoi*[2] is also probably identical with this fraction.

The physical constants and the *n/d* plot of fraction 5, the second sesquiterpene, indicate longifolene, and this was confirmed by the preparation of the hydrochloride.[13] This sesquiterpene was also identified in the volatile oil of *D. colensoi*.[2]

The physical constants of the third sesquiterpene, fraction 6, are in close agreement with those reported for aromadendrene,[14] and on ozonolysis aromadendrone was obtained in good yield.

Fraction 7 is the sesquiterpene (−)-metrosiderene, first reported in the volatile oil of *Metrosideros umbellata*.[15] This sesquiterpene may be characterized by a crystalline nitrosate. The identity of this sesquiterpene with that from *M. umbellata*, was established by a comparison of the physical constants,[16] and by the identity of the infra-red spectra.

The sesquiterpene, fraction 8, gave cadinene dihydrochloride in moderate yield (50%). It is probably one of the isomeric forms of cadinene, and from the optical rotation it would appear

to be (+)-γ-cadinene.[17] The sesquiterpene, fraction 9, gave a quantitative yield of cadinene dihydrochloride, and the physical constants identify it as (+)-δ-cadinene.[17] Following the cadinene fractions, a small, impure liquid sesquiterpene alcohol fraction, fraction 10, and then a sesquiterpene alcohol fraction 11, which solidified in the column, were obtained. The distillation was stopped at this point and a total of 50 mg. of solid, m.p. 136–137°, was collected from the still head. This alcohol probably contains a tetrasubstituted double bond, since although it is unsaturated (positive test with tetranitromethane) it is resistant to hydrogenation with Adams's platinum oxide catalyst in ethanol. It failed to yield a crystalline 3 : 5-dinitrobenzoate, or hydrochloride. The molecular formula, $C_{15}H_{26}O$, is consistent with a mono-unsaturated dicyclic structure, and the reactions correspond with those described for torreyol, m.p. 139–140°,[18] but this could not be confirmed since no authentic specimen was available for comparison.

The solid which separated from the distillation residue was identified as (+)-phyllocladene, by mixed melting point and by the preparation of the hydrochloride m.p. 106°. After removal of the phyllocladene, the filtrate was separated into five fractions by distillation, and α-camphorene was identified, albeit in poor yield, in the fraction b.p. 160–170°/3·5 mm., by the preparation of its tetrahydrochloride.[19] Fractional distillation of the remainder of the residue revealed that it was a very complex mixture of substances, none of which was present in any appreciable concentration.

There are notable differences in the composition of the terpene fraction of this essential oil compared with that isolated from the other members of the genus. These are emphasized in the following table.

	D. biforme	*D. colensoi*[2]	*D. kirkii*[1]	*D. cupressinum*[20]
α-Pinene	absent	17%	65%	12%
Myrcene	12%	4%	4%	0·5%
Limonene } Dipentene }	absent	5%	2%	2%

The sesquiterpene fractions of the *Dacrydium* oils show a general similarity. The *n/d* diagram for this oil (Fig. 1) shows a striking resemblance to that described by Briasco & Murray[2] for *D. colensoi*, which oil probably contains all the sesquiterpenes present in this one.

Preparation and characterization of fractions

Leaves and terminal branchlets of *D. biforme* were collected in September from trees growing on Leith Saddle, near Dunedin. These were steam-distilled in 250-lb. batches, extraction being complete after 20–24 hours. The oil (1690 c.c., 0·34% v/w) was divided into two fractions, (*a*) b.p. 64–120°/2·5–15 mm. (990 c.c.), and (*b*) 120°/2·5 mm. (700 cc.), by distillation in a Claisen flask. The lower-boiling fraction was then fractionated through a Lecky–Ewell column in the usual manner at a reflux ratio of 25 : 1 and take-off rate of 2–3 c.c./hour. Fraction 11 crystallized in the still-head and prevented further operation of the column, and the residue in the stillpot was added to the diterpene fraction which had partially solidified.

Fraction 1.—The identity of this fraction with myrcene was confirmed through the preparation of the adduct with maleic anhydride, b.p. 177–180°/3 mm., and m.p. 34°. After saponification, acidification, and repeated crystallization from acetonitrile the adduct gave an acid, m.p. 122°. Diels & Alder[20] gave a m.p. 122–123° for the pure acid from the myrcene adduct.

Fraction 2.—Bromination with bromine in pentyl alcohol gave β-terpinene tetrabromide, m.p. 151°, after repeated crystallization from ethanol, undepressed on admixture with an authentic specimen. This fraction obviously contained another terpene as the yield of tetrabromide was poor (10%), and the physical constants differ somewhat from those recorded for β-terpinene.[9]

Fraction 3.—Three small fractions 3a, n_D^{20} 1·4653, d_{20}^{20} 0·9078; 3b, n_D^{20} 1·4860, d_{20}^{20} 0·9491; and 3c, n_D^{20} 1·4870, d_{20}^{20} 0·8960, each less than 1 c.c., have been listed as fraction 3 in Table I. The high densities of 3a and 3b indicate oxygenated terpenes and this was confirmed by elementary analysis. However, it was impossible to obtain satisfactory analyses because of the impurity of the fractions. The infra-red spectrum of 3c showed that it was an ester. In each case the small volume precluded further investigation of the fraction.

Fraction 4.—This hydrocarbon fraction could not be identified with any known sesquiterpene. No crystalline hydrochloride could be prepared. The molecular refraction (64·6) indicates a tricyclic sesquiterpene. Dehydrogenation of the fraction by means of selenium yielded cadalene (b.p. 118°/3 mm.), which gave a picrate, m.p. 115°, undepressed on admixture with an authentic specimen.

Fraction 5.—The physical constants of this fraction agree closely with those of (+)-longifolene. Identity with this compound was confirmed through the preparation of a monohydrochloride (needles from ethanol), m.p. and mixed m.p. with longifolene hydrochloride 58°.

Fraction 6.—The fraction in glacial acetic acid gave, after ozonolysis, a neutral fraction b.p. 144–152°/3·5 mm., which solidified and had m.p. 83–84° after crystallization from aqueous methanol, and was undepressed by an authentic sample of aromadendrone. The oxime[15] had m.p. 103°.

Fraction 7.—The physical constants of this sesquiterpene were very similar to those already reported for (−)-metrosiderene, and a comparison of the infra-red spectra revealed that this fraction was (−)-metrosiderene. The sesquiterpene yielded a crystalline nitrosate, m.p. 122° (decomp.).

Fraction 8.—This sesquiterpene was identified as one of the isomers of cadinene through the preparation of a dihydrochloride (50% yield), m.p. and mixed m.p. with cadinene dihydrochloride 116–117°. Dehydrogenation of the fraction by means of selenium yielded cadalene (b.p. 118°/3 mm.), which gave a picrate, m.p. and mixed m.p. 115°.

Fraction 9.—The physical constants of this fraction are in close agreement with those reported for (+)-δ-cadinene.[17] Identity with this compound was confirmed through the preparation of a dihydrochloride, m.p. and mixed m.p. with cadinene dihydrochloride 116–117°. Dehydrogenation of the fraction by means of selenium yielded cadalene (b.p. 118°/3 mm., n_D^{20} 1·5), which gave a picrate, m.p. and mixed m.p. 115°, and a trinitrobenzene derivative, m.p. 122°.

Fraction 11.—This solid sesquiterpene alcohol (50 mg.) formed feathery needles m.p. 136° after crystallization from ethanol (Found C, 80·90; H, 11·60. Calculated for $C_{15}H_{26}O$ C, 81·02; H, 11·79%). No crystalline hydrochloride was obtained, nor could a crystalline 3 : 5-dinitrobenzoate be prepared. It gave a positive test for unsaturation with a chloroform solution of tetranitromethane.

Diterpene fraction.—This fraction (700 c.c.) after keeping in the ice-chest for one month deposited phyllocladene, m.p. 95–96° after repeated crystallization from ethanol, undepressed by an authentic specimen of (+)-phyllocladene. *iso*Phyllocladene was apparently absent. With hydrogen chloride in dry ether, phyllocladene yielded a crystalline hydrochloride, m.p. 106°, undepressed by an authentic specimen of phyllocladene hydrochloride.

After removal of the phyllocladene, a small portion of the residue was distilled in a semi-micro fractionating column of 42 theoretical plates. No pure compound could be isolated and the steady drift in the physical constants of successive distillate aliquots merely served to emphasize the great complexity of the mixture. The bulk of the residue was then divided into a series of fractions by distillation in a Claisen flask. Some of the fractions deposited small amounts of phyllocladene, while one fraction, b.p. 160–170°/3·5 mm., gave a crystalline hydrochloride, m.p. 131°, when treated with hydrogen chloride in anhydrous ether. This was identified as α-camphorene tetrahydrochloride, by mixed melting point with an authentic specimen.

Acknowledgments

This work has been assisted by grants from the Research Fund of the University of New Zealand and from the Mellor Research Fund. Analyses are by Dr. A. D. Campbell of this Department.

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Received 25 April, 1955

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THE EFFECT OF FEEDING DIFFERENT SEAWEED MEALS ON THE MINERAL AND NITROGEN METABOLISM OF LAYING HENS

By C. J. E. HAND* and C. TYLER

Three different seaweed meals, *Laminaria cloustoni*, *Ascophyllum nodosum* and *Laminaria saccharina*, were fed to poultry in three different experiments. In each experiment levels of 10% and 20% in a basal ration were compared with the basal ration itself.

When the seaweed was low in energy the birds showed loss in weight or lowered egg production or both. The high chloride intake when the ration contained seaweed resulted in a greatly increased water intake and a heavy excretion of both chloride and water, but this did not appear to affect the general health of the birds. Changes observed in calcium, phosphorus and nitrogen retentions and balances could be explained on the basis of changes in egg production. The seaweeds had no effect on the chemical composition of egg content and shell, nor on porosity and shell thickness. On the basis of these criteria seaweed meals therefore had no beneficial effects and at the higher level of 20% were detrimental in some respects.

In a further, long-term feeding experiment *Laminaria cloustoni* (stipe), *Laminaria cloustoni* (frond) and *Ascophyllum nodosum* were fed at levels of 10% and 15%, each level being given for 100 days. The results, as far as they were comparable, supported the more detailed balance experiments.

Introduction

From time to time the question has arisen as to whether seaweed could usefully be fed to poultry, and Miller & Bearse,[1] Sumita, Kuwabata & Fujoka,[2] Batt[3] and Black[4] have reported feeding experiments with seaweed. The general position seems to be that poultry show no adverse effects when reasonable quantities are fed: the palatability of the ration may be improved and there is an increase in the iodine content of the egg. On the other hand, Lunde[5] suggested that the high chloride content of seaweed meal is probably a limiting factor in its use.

As far as the authors are aware, no balance experiments have been carried out using rations containing seaweed meals and it was therefore decided to carry out such experiments, determining the balance of calcium, phosphorus, chloride and nitrogen, while at the same time recording more general data about the birds and their eggs.

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Three types of seaweed meal, namely, *Laminaria cloustoni*, *Ascophyllum nodosum* and *Laminaria saccharina*, were used and this paper deals with experiments covering all three types.

Experimental

Birds

Four Rhode Island Red pullets were obtained fresh from the University Farm for each experiment. The pullets were in lay or just about to lay when put into the cages and the experiment began when all were laying regularly. Each bird was housed separately in the usual type of poultry metabolism cage.

Plan of the experiments

Table I shows the plan of the experiments, 10% and 20% levels of the appropriate seaweed being introduced at different times with adequate control periods (C) before and after each seaweed feeding period (S). There were thus five periods which have been named C1, S2, C3, S4 and C5.

Table I

General plan of the experiments

Period	Symbol	Daily regime
Control	C1	120 g. of basal ration
Seaweed	S2	108 g. „ „ „ + 12 g. of seaweed meal
Control	C3	120 g. „ „ „
Seaweed	S4	96 g. „ „ „ + 24 g. of „ „
Control	C5	120 g. „ „ „

In each case a mineral supplement of 5 g. of calcium carbonate and 0·5 g. of sodium chloride was given.

Duration of the experiments

The first experiment lasted 70 days, i.e., 14 days per period, whilst the second and third experiments lasted 80 days, i.e., 16 days per period.

Time of the experiments

Experiment 1 was carried out in May, June and July, Experiment 2 in June, July and August and Experiment 3 in April, May and June.

Ration

The basal ration used throughout consisted of 39 parts of middlings; 34 parts of barley meal; 18 parts of crushed oats; 18 parts of bran; 12 parts of maize gluten meal; 6 parts of fish meal and 1 part of cod liver oil. Different consignments of the ration naturally varied on analysis but not to a very great extent and Table II shows the average analysis for all three experiments. In the same table the analysis of the seaweeds is also shown.

Table II

Average analysis of the dry matter of the basal ration and of the three seaweed meals

	Basal ration %	*Laminaria cloustoni* %	*Ascophyllum nodosum* %	*Laminaria saccharina* %
Crude protein	18·59	14·12	10·25	4·15
Ether extract	3·62	1·30	1·90	0·31
Crude fibre	8·48	5·40	3·50	4·80
Nitrogen-free extract	64·16	37·90	60·85	72·54
Total ash	5·15	41·28	23·50	18·20
Insoluble ash	0·53	0·54	0·32	0·21
Calcium	0·50	1·21	1·26	1·48
Phosphorus	0·94	0·36	0·12	0·13
Chloride	0·15	13·82	5·56	5·95
Moisture	13·41	7·20	10·80	8·18

During the control periods 120 g. of the basal ration were mixed with 5 g. of calcium carbonate and 0·5 g. of sodium chloride, and the mixture then made into a crumbly mash by the

addition of 100 ml. of water. This constituted the daily ration with drinking water *ad lib.* In the two seaweed feeding periods of each experiment the appropriate quantity of seaweed meal replaced a corresponding weight of basal ration, to produce respectively rations containing 10 and 20% of seaweed. The resulting 120 g. were then mixed with calcium carbonate, sodium chloride and water as before. In each case the seaweed had been dried and ground to pass a 60-mesh sieve. *Laminaria cloustoni* was given in the first experiment, *Ascophyllum nodosum* in the second and *Laminaria saccharina* in the third.

The food was fed at 9.30 a.m. each morning and fresh distilled water was given. At the same time food and water residues were collected and measured, along with droppings, eggs and feathers, if any. Care was taken to see that adequate drinking water was always available and records of all supplies were kept.

Methods of analysis

The analytical methods for calcium, phosphorus, chloride and nitrogen were, with only slight modifications, the same as those described by Tyler.[6]

Other measurements

Shell thickness was measured on five pieces of true shell (Tyler & Geake[7]) using a micrometer screw gauge and the mean taken. Porosity coefficients for the eggs were obtained using the method of Tyler.[8]

Treatment of the data

Individual daily intake and output values and individual egg measurements were made, and then the mean daily value for each period, namely, three control and two seaweed periods, was calculated for each bird. It was these daily means for four birds and five periods which were used for the analysis of variance. However, the amount of data is so great that in the tables, only the general period means each covering the four birds are shown, i.e., bird-day means. When calculating significant differences the bird-day mean for a seaweed period was always compared with the mean of the two control period means which came immediately before and after this seaweed period. Thus period S2 was compared with the mean of C1 and C3 designated period C_{13} and period S4 with the mean of C3 and C5, designated period C_{35}.

In this way it was hoped to reduce the effects, if any, caused by temporal changes.

Significant differences are shown in the usual way : N.S., *, ** and *** represent, respectively, not significant, significant at $P < 0.05$, at $P < 0.01$ and at $P < 0.001$.

Occasionally reference will be made to data for sub-periods, each of which represents half a full period, and also to daily data, but none of these values is given except where they are necessary to amplify the discussion.

Definition of terms

Retention.—By ' retention ' is meant the difference between the intake of a substance and the output of that substance in the droppings. The use of this term is made necessary by the fact that the term ' absorption ' cannot be used since poultry excrete urine and faeces together.

Balance.—The term ' balance ' will therefore refer to the intake of a substance minus the total outgo of that substance including eggs :

i.e., Balance = Intake − (outgo in droppings + eggs)

Clearly, retention and balance will be the same if no eggs are laid, and retention minus outgo in eggs will give the balance figure :

i.e., Balance = Retention − outgo in eggs

Results

General health of the birds

All the birds in Experiments 1 and 2 remained in good health throughout the whole of the experiment. During control periods droppings were normal, but during seaweed periods they became greener and also more watery. The watery consistency was caused by the consumption

of larger quantities of water, as will be seen later. Despite this and the increased intake of chloride there was no deterioration in health.

The same can be said for birds 1, 2 and 3 in Experiment 3, but bird 4 was the exception. She appeared healthy in periods C1 and S2, egg production began to decrease in period C3 and food consumption suddenly dropped at the beginning of period C5 and she died on day 75 of the experiment. A post-mortem examination revealed extensive lymphomatosis. Since this condition may have begun to develop earlier than was suspected, it was decided not to use the data for her in the statistical analysis, and therefore for Experiment 3 the results refer to three birds only.

With regard to the shedding of feathers, this was only of a measurable degree with the birds in Experiment 2. It began around the beginning of period S4 and continued to the end of the experiment and may have been caused by the 20% level of seaweed, or the high temperatures obtaining during period C3 or a combination of both.

Food intake

If all the food fed was consumed then the intakes of the constituents under investigation were as shown in Table III. From these values it will be observed that between experiments, the control rations showed very little variation. Further, it is seen that, in each case, the addition of seaweed slightly increased the amount of calcium and decreased the amount of phosphorus and nitrogen and that chloride values were most affected, a considerable increase occurring in Experiments 2 and 3 and a very great increase in Experiment 1.

Table III

Amounts of calcium, phosphorus, chloride and nitrogen present in the daily rations (g.)

	Ca	P	Cl	N
Experiment 1				
Controls C1, C3, C5	2·53	1·01	0·42	3·03
Seaweed S2 10%	2·61	0·95	1·95	2·98
Seaweed S4 20%	2·68	0·89	3·48	2·92
Experiment 2				
Controls C1, C3, C5	2·48	0·97	0·46	3·06
Seaweed S2 10%	2·56	0·88	1·04	2·95
Seaweed S4 20%	2·64	0·80	1·62	2·84
Experiment 3				
Controls C1, C3, C5	2·42	0·96	0·45	3·19
Seaweed S2 10%	2·54	0·88	1·09	2·95
Seaweed S4 20%	2·66	0·80	1·73	2·71

With regard to actual consumption, all the birds in Experiments 1 and 2 ate the whole of their ration each day and in Experiment 3 birds 1, 2 and 3, the only ones to be considered, also ate all their food. Throughout each of the experiments it was noticed that the birds ate their food far more eagerly when it contained seaweed, which suggests that it had a greater palatability.

Water consumption

This is defined as the amount of water drunk plus the amount consumed with the wet food, but, of course, this latter addition is an almost constant quantity. Table IV shows the results and it is quite clear that seaweed feeding increased the water consumption, the increase being significant in all except one case. Further, the increase was always greater with the greater quantities of seaweed. The increased intake was related to chloride content, the largest increases occurring in Experiment 1 where the seaweed highest in chloride was fed.

The high value for water consumption in period C3 of Experiment 2 compared with periods C1 and C5 was almost certainly caused by the high temperature prevailing at that time. It should also be pointed out that individual birds showed considerable variations in water consumption and that one bird receiving the highest levels of chloride actually consumed over 1000 g. of water on certain days.

Table IV

Water consumption per period (g.). Bird means

Period	Expt. 1	Expt. 2	Expt. 3
C1	429	480	337
S2	619	557	434
C3	484	583	350
S4	904	672	565
C5	492	495	373
$S2 - C_{13}$	+ 163*	+ 26 N.S.	+ 91**
$S4 - C_{35}$	+ 416***	+ 133*	+ 204***

Live weight changes

The bird means for changes in live weight are recorded in Table V.

Table V

Live weight changes per period (g.). Bird means

Period	Expt. 1	Expt. 2	Expt. 3
C1	− 42	− 7	− 89
S2	− 71	− 16	− 97
C3	+ 57	+ 58	+ 1
S4	− 156	− 120	− 42
C5	+ 99	+ 84	+ 22
$S2 - C_{13}$	− 79 N.S.	− 42 N.S.	− 53 N.S.
$S4 - C_{35}$	− 234***	− 191*	− 54 N.S.

In Experiment 1 the feeding of 10% seaweed caused a considerable decrease in live weight but this change was not significantly different from the change in control periods C1 and C3. However, this was caused entirely by the behaviour of bird 1, and if the values for birds 2, 3 and 4 only were taken then the result was a highly significant loss in period S2 compared with the average for period C_{13}. At the 20% level of seaweed feeding, the loss in weight was highly significant. This reduction in live weight may be explained as follows. The ration fed during the control periods had a calculated starch equivalent sufficient for maintenance and about 70% egg production, according to the requirements quoted by Halnan.[9] The addition of seaweed probably resulted in a lowering of the starch equivalent. At the lower level of seaweed feeding the birds generally maintained the same level of egg production as in the control periods (see section on Egg production) but suffered a loss in weight, whilst at the higher level of seaweed feeding, which would mean an even lower starch equivalent, the birds showed a greater loss in body weight and also a reduced egg production.

In Experiment 2, losses in weight occurred in the seaweed periods but only the 20% level caused a significant decrease. With individual birds the decrease was greater if egg production was maintained. The seaweed used in this experiment, *Ascophyllum nodosum*, also had a composition such that its substitution for part of the ration would probably reduce the starch equivalent intake, so that the explanation of the live-weight changes may be the same as for Experiment 1.

The results for Experiment 3 show that although losses in weight occurred when seaweed was given, yet they were not significantly different from the changes occurring in the control periods. The seaweed used, *Laminaria saccharina*, had a much higher laminarin content (26·5%) than the other two seaweeds (0·4% and 3·5%, respectively) and it may be that this seaweed did not greatly reduce the starch equivalent intake and therefore did not lead to significant weight losses. It certainly did not interfere with egg production as greatly as did the first two meals.

Egg production

The results for Experiment 1 (Table VI) show that the 10% level of seaweed feeding did not produce any significant effect on egg production, but that the 20% level caused a significant reduction. As suggested in connexion with live-weight changes, this reduction coupled with weight losses may have been caused by the lowered starch equivalent of this ration.

Table VI

Egg production per period. Bird means

Period	Expt. 1	Expt. 2	Expt. 3
C1	10·50	10·00	11·67
S2	9·00	7·50	11·33
C3	8·75	7·50	10·67
S4	5·75	4·75	9·00
C5	7·25	1·75	10·67
$S2 - C_{13}$	− 0·63 N.S.	− 1·25 N.S.	+ 0·16 N.S.
$S4 - C_{35}$	− 2·25*	+ 0·13 N.S.	− 1·67 N.S.

In Experiment 2 only bird 3 remained in lay throughout the whole of the experiment, and generally there was a pronounced decline in egg production as time went on. It may be that here the 20% level of seaweed produced an effect on egg production which for some unknown reason carried over into the control period C5, or alternatively there may have been a natural seasonal decline.

However, none of the differences was significant.

In Experiment 3 there was quite a high egg production throughout and no differences were significant. This again supports the concept advanced earlier that this seaweed with high laminarin content did not greatly reduce the starch equivalent intake and hence did not interfere with egg production.

The values for mean egg weight have not been given, for no differences were significant apart from a significant decrease when 10% seaweed was fed in Experiment 1.

Calcium

The values for calcium retention and balance are shown in Table VII.

Table VII

Calcium retention and balance (mg.). Bird-day means

Period	Expt. 1		Expt. 2		Expt. 3	
	Retention	Balance	Retention	Balance	Retention	Balance
C1	1178	− 119	1094	+ 24	1335	− 139
S2	1068	− 48	879	+ 100	1314	− 90
C3	1046	− 35	910	+ 82	1308	− 1
S4	797	+ 126	649	+ 134	1206	+ 123
C5	933	+ 35	429	+ 235	1265	+ 45
$S2 - C_{13}$	− 44 N.S.	+ 19 N.S.	− 123 N.S.	+ 47 N.S.	− 8 N.S.	− 20 N.S.
$S4 - C_{35}$	− 193*	+ 126*	− 21 N.S.	− 25 N.S.	− 81*	+ 101 N.S.

In Experiment 1 there was a tendency for calcium retention to decline throughout the experiment as far as the end of period S4, and it then showed a slight recovery. There was, however, a significant difference when 20% seaweed was fed, the retention of calcium being considerably lower than the corresponding control periods. The balances were at first negative and later positive and the balance was significantly higher in the S4 period than in the C_{35} period. Before drawing any conclusions here about the effect of seaweed on calcium metabolism, it is important to note that Tyler[10] has shown that, over a period, there is a close relationship between calcium secreted as shell and calcium retained. Since egg production varied in this experiment and fell in the same way as the calcium retention, it is therefore probable that these changes in retention were simply a reflection of changes in egg production and hence in the amount of calcium lost from the body as shell. The relationship mentioned above is not such as to produce a constant balance and hence there is nothing in the change in balance to negative this concept.

There were no significant differences for either retention or balance in Experiment 2.

The results for Experiment 3 followed fairly closely on the lines of Experiment 1. However, any changes which did arise could be readily explained on the basis outlined above.

Phosphorus

From the results presented in Table VIII, it will be seen that in no experiment were there any significant differences either in retention or balance. The trends which actually occurred appear to have been caused by two sets of circumstances, firstly the lowered intake in the seaweed periods and secondly the increased excretion associated with shell formation. Both these factors will decrease retention and the small fluctuations in retention are thus readily explained.

Table VIII

Phosphorus retention and balance (mg.). Bird-day means

Period	Expt. 1		Expt. 2		Expt. 3	
	Retention	Balance	Retention	Balance	Retention	Balance
C1	83	+ 2	92	+ 19	80	− 11
S2	91	+ 22	90	+ 35	73	− 11
C3	96	+ 30	95	+ 39	99	+ 21
S4	95	+ 54	82	+ 47	88	+ 23
C5	93	+ 39	136	+ 123	99	+ 26
$S2 - C_{13}$	+ 1 N.S.	+ 6 N.S.	− 4 N.S.	+ 6 N.S.	− 17 N.S.	− 16 N.S.
$S4 - C_{35}$	+ 1 N.S.	+ 19 N.S.	− 34 N.S.	− 34 N.S.	− 11 N.S.	− 1 N.S.

Chloride

In the case of chloride it will be remembered that there is a considerable increase in chloride intake when seaweed is fed, and this must be borne in mind when discussing the results.

The results (Table IX) are consistent for all three experiments. In every case there was a highly significant increase in the retention of chloride when seaweed was fed. Compared with their respective controls the higher level of seaweed feeding gave the greater increase in retention. It is also of interest to note that the retentions in periods C3 and C5 were considerably less than those in C1, and it would appear that during these periods the birds were getting rid of extra chloride which their tissues were compelled to store in the previous high-chloride periods.

Table IX

Chloride retention and balance (mg.). Bird-day means

Period	Expt. 1		Expt. 2		Expt. 3	
	Retention	Balance	Retention	Balance	Retention	Balance
C1	75	0	65	+ 2	73	− 1
S2	108	+ 41	76	+ 26	94	+ 24
C3	29	− 36	33	− 16	44	− 19
S4	94	+ 51	61	+ 26	84	+ 31
C5	21	− 32	− 17	− 30	25	− 34
$S2 - C_{13}$	+ 56***	+ 59**	+ 27***	+ 33***	+ 35***	+ 34***
$S4 - C_{35}$	+ 69***	+ 85***	+ 53***	+ 49***	+ 49***	+ 58***

The balance values strongly support the ideas expressed above, since it is clear that in periods C3 and C5 the birds went into negative chloride balance. All the differences are highly significant.

The daily values (not given) showed very distinctly that the reaction to higher chloride intake was immediate, a greater water consumption and a greater chloride excretion occurring at once.

Nitrogen

It is apparent from the results in Table X that there was a significant fall in retention during the feeding of both levels of seaweed in Experiment 1 and with the higher level of feeding in Experiment 3. In the other cases there was also a fall but it was not significant.

The balance results indicate that none of the results was significant and that in some cases seaweed gave an increased, and in some a decreased, balance compared with the controls.

Seaweed feeding altered the intake of nitrogen in all experiments, but these changes were insufficient to account for the changes in retention. The lowered retention appears to be, in part, associated with lowered egg production and the balance data tend to support this. Losses

Table X

Nitrogen retention and balance (mg.). Bird-day means

Period	Expt. 1		Expt. 2		Expt. 3	
	Retention	Balance	Retention	Balance	Retention	Balance
C1	803	+ 15	677	+ 28	820	− 10
S2	657	− 12	506	+ 20	748	− 40
C_3	659	− 8	561	+ 16	793	+ 17
S4	513	+ 93	356	+ 18	630	+ 30
C_5	564	+ 11	213	− 90	751	+ 62
$S_2 - C_{13}$	− 74*	− 16 N.S.	− 113 N.S.	− 2 N.S.	− 59 N.S.	− 44 N.S.
$S_4 - C_{35}$	− 99**	+ 91 N.S.	− 31 N.S.	+ 55 N.S.	− 142*	− 10 N.S.

of feathers in some instances may cloud the issue but, as with calcium, there appears to be a relation between retention of nitrogen and nitrogen in the egg.

Egg composition

It is evident from Table XI that, with the exception of one result, the feeding of none of the three seaweeds at either level of intake had any significant effect on the percentage of calcium, phosphorus, chloride and nitrogen in the egg content. Further, only in the case of chloride were the changes always in the same direction. With chloride, the content in every case showed a greater percentage when seaweed was fed, and in one, but only one, case was it significant.

Table XI

Percentage calcium, phosphorus, chloride and nitrogen in egg content and percentage calcium, phosphorus and chloride in nitrogen-free shell

Period	Egg content				Shell		
	Ca	P	Cl	N	Ca	P	Cl
			Expt. 1				
C1	0·061	0·193	0·184	1·84	38·4	0·176	0·074
S2	0·057	0·192	0·192	1·87	38·4	0·168	0·074
C_3	0·057	0·186	0·189	1·84	38·4	0·166	0·071
S4	0·059	0·182	0·201	1·86	38·4	0·157	0·088
C_5	0·056	0·188	0·190	1·87	38·3	0·153	0·065
$S_2 - C_{13}$	−0·002 N.S.	+0·003 N.S.	+0·006 N.S.	+0·03 N.S.	0·0 N.S.	−0·003 N.S.	+0·002 N.S.
$S_4 - C_{35}$	+0·003 N.S.	−0·005 N.S.	+0·012**	+0·01 N.S.	+0·1 N.S.	−0·003 N.S.	+0·020*
			Expt. 2				
C1	0·052	0·215	0·192	1·88	38·3	0·166	0·064
S2	0·059	0·214	0·198	1·88	38·4	0·164	0·076
C_3	0·058	0·212	0·188	1·91	38·3	0·164	0·063
S4	0·056	0·211	0·210	1·82	38·3	0·166	0·085
C_5	0·055	0·201	0·221	1·83	38·2	0·161	0·072
$S_2 - C_{13}$	+0·004 N.S.	+0·001 N.S.	+0·008 N.S.	−0·02 N.S.	+0·1 N.S.	−0·001 N.S.	+0·013 N.S.
$S_4 - C_{35}$	−0·001 N.S.	+0·004 N.S.	+0·006 N.S.	−0·05 N.S.	+0·1 N.S.	+0·004 N.S.	+0·018 N.S.
			Expt. 3				
C1	0·061	0·214	0·179	1·96	38·4	0·149	0·043
S2	0·061	0·208	0·179	1·97	38·3	0·154	0·042
C_3	0·062	0·205	0·174	1·96	38·3	0·159	0·036
S4	0·062	0·209	0·179	1·97	38·3	0·165	0·044
C_5	0·062	0·207	0·174	2·01	38·3	0·165	0·038
$S_2 - C_{13}$	−0·001 N.S.	−0·002 N.S.	+0·003 N.S.	+0·01 N.S.	−0·1 N.S.	0·000 N.S.	+0·003 N.S.
$S_4 - C_{35}$	0·000 N.S.	−0·003 N.S.	+0·005 N.S.	−0·02 N.S.	0·0 N.S.	+0·003 N.S.	+0·007 N.S.

A similar picture arises when the percentage composition of the nitrogen-free shell is considered. Calcium, phosphorus and chloride values show only one significant change, namely, in an increase of chloride on the 20% level of seaweed in Experiment 1. However, it should again be observed that in every case, the shell chloride did increase when seaweed was fed, and it may be caused by more adventitious chloride being picked up by the shell on its way through the cloaca when the droppings were rich in chloride.

Porosity and shell thickness

There were no significant differences in any experiment in the porosity and thickness of the shells of the eggs (Table XII).

Table XII

Shell thickness (μ) and porosity coefficients (mg. loss/sq.cm./day)

Period	Expt. 1		Expt. 2		Expt. 3	
	Thickness	Porosity	Thickness	Porosity	Thickness	Porosity
C_1	307	2·41	304	2·33	337	1·74
S_2	312	2·58	303	2·56	337	1·71
C_3	302	2·27	307	2·72	336	1·72
S_4	291	2·58	301	2·78	336	1·75
C_5	315	2·45	305	2·46	333	1·56
$S_2 - C_{13}$	+ 8 N.S.	+ 0·24 N.S.	− 3 N.S.	+ 0·04 N.S.	+ 1 N.S.	− 0·02 N.S.
$S_4 - C_{35}$	− 18 N.S.	+ 0·22 N.S.	− 5 N.S.	+ 0·19 N.S.	+ 2 N.S.	+ 0·11 N.S.

Long-term feeding experiment

Having established certain facts by means of balance experiments it was then decided to run an experiment with more birds and for a longer time. This experiment will only be described briefly.

Group-feeding trials are not very satisfactory because there is no control of individual feeding and the interpretation of the results is not easy. The experiment was therefore planned as a randomised block using four birds (Rhode Island Red × Light Sussex) as replicates for each of four treatments. Part I of the experiment lasted 100 days during which time Group A received the control ration (150 g. of layer's mash, 6·5 g. of calcium carbonate, 0·6 g. of sodium chloride and 100 ml. of water) ; Group B had 15 g. of layer's mash replaced by 15 g. of *Laminaria cloustoni* (stipe), i.e., 10% seaweed. Similarly, Group C received 10% *Laminaria cloustoni* (frond) and Group D 10% of *Ascophyllum nodosum*. In Part II of the experiment the control birds received the same ration as before but the others now received 15% seaweed. The analysis of the basal ration and the three seaweeds is given in Table XIII.

Table XIII

Analysis of the dry matter of the basal ration and of the seaweed meals

	Basal ration	Seaweed meals		
		B	C	D
	%	%	%	%
Crude protein	19·4	10·9	7·4	9·1
Ether extract	4·6	0·5	0·5	2·3
Crude fibre	7·1	10·3	5·7	4·0
Nitrogen-free extract	63·7	43·4	66·3	57·1
Total ash	5·2	34·9	20·1	27·5
Insoluble ash	0·4	1·0	0·9	0·5
Calcium	0·75	1·78	1·64	1·58
Phosphorus	1·03	0·27	0·17	0·15
Chloride	0·18	10·04	6·58	5·98
Moisture	11·3	10·6	11·3	12·6
Starch equivalent	65·6	—	—	—
Digestible protein	13·9	—	—	—

Seaweed meals B : *Laminaria cloustoni* (stipe)
C : *Laminaria cloustoni* (frond)
D : *Ascophyllum nodosum*

All the birds were individually fed once a day with accurately weighed quantities of food, and daily water consumption and egg production were recorded. Live-weight changes over the periods were also noted. The routine was similar to the balance experiments except that droppings and eggs were not analysed. Results are shown in Tables XIV and XVI.

The results were analysed statistically for Parts I and II of the experiment separately and the main findings were as follows :

1. The general health of the birds did not seem to be affected by feeding seaweed and there was some indication that seaweed improved palatability.

2. The control ration gave live-weight gains in both parts of the experiment, whereas *Laminaria cloustoni* (stipe) gave significant losses at both levels of feeding when compared with the control. *Laminaria cloustoni* (frond) and *Ascophyllum nodosum* did not show significant losses of live weight at the 10% level but did so at 15% (Table XIV).

3. None of the seaweed meals significantly affected egg production at either level of feeding (Table XV).

Table XIV

Live-weight changes in the individual birds (g.)

Part I: 10% seaweed

Block	Treatment A	B	C	D
1	+ 47	− 33	− 79	− 153
2	+ 141	− 113	− 81	− 1
3	+ 187	− 101	+ 125	+ 32
4	+ 214	− 161	+ 95	+ 179
Mean	+ 147	− 102	+ 15	+ 14
Difference B − A		−249**		
C − A			− 132 N.S.	
D − A				− 133 N.S.

Part II: 15% seaweed

Block	Treatment A	B	C	D
1	+ 177	− 190	− 115	+ 9
2	+ 20	− 137	+ 48	− 258
3	+ 101	− 32	− 175	− 280
4	+ 175	+ 39	+ 1	− 180
Mean	+ 118	− 80	− 60	− 177
Difference B − A		− 198*		
C − A			− 178*	
D − A				− 295**

Table XV

Total egg production per bird

Part I: 10% seaweed

Block	Treatment A	B	C	D
1	73	64	79	69
2	76	82	85	62
3	78	81	87	77
4	71	69	74	72
Mean	75	74	81	70
Difference B − A		− 1 N.S.		
C − A			+ 6 N.S.	
D − A				− 5 N.S.

Part II: 15% seaweed

Block	Treatment A	B	C	D
1	45	42	61	48
2	67	84	63	27
3	76	70	76	73
4	74	56	68	72
Mean	66	63	67	55
Difference B − A		− 3 N.S.		
C − A			+ 1 N.S.	
D − A				− 11 N.S.

4. The greatest effect was seen in increased water consumption, which, in turn, produced very watery droppings. At the 10% level of feeding all three seaweeds caused a significant increase in water consumption over the control group, and at the 15% level this was even further increased (Table XVI).

Table XVI

Mean daily water consumption per bird (g.)

Part I: 10% seaweed

Block	Treatment			
	A	B	C	D
1	344	431	544	386
2	371	405	434	504
3	260	474	381	381
4	312	417	450	409
Mean	322	432	452	420
Difference B — A		+ 110*		
C — A			+ 130**	
D — A				+ 98*

Part II: 15% seaweed

Block	Treatment			
	A	B	C	D
1	410	598	604	518
2	429	533	567	464
3	305	669	592	552
4	332	582	634	681
Mean	369	596	599	554
Difference B — A		+ 227**		
C — A			+ 230**	
D — A				+ 185**

Conclusions

From the balance experiments it is apparent that none of the seaweed meals considered produced any positive results of value, although *Laminaria saccharina* showed no detrimental effects.

The chief disadvantage under the condition of these experiments seemed to be that, when *Laminaria cloustoni* or *Ascophyllum nodosum* was fed, the birds were receiving less energy than was contained in the normal ration. Thus the birds tended to lose weight or show a fall in egg production or both, depending upon the type of seaweed and the level fed. The high chloride content of all three seaweed meals caused very heavy water consumption and heavy excretion of water and chloride, but neither this nor any other factor appeared to affect the health of the birds although it cannot be ruled out that the high chloride content of the seaweed meal may have had some adverse influence on live weight and egg production.

Any changes which were observed in the calcium, phosphorus and nitrogen retention and balance could generally be explained on the basis of changes in egg production, whilst the seaweed appeared to have no effect on egg content or shell composition, or on porosity or shell thickness.

In the long-term experiment, all three seaweeds when fed at the 15% level caused live-weight losses, again probably owing to an insufficiency of energy, but at the 10% level only *Laminaria cloustoni* (stipe) caused live-weight losses. There was no obvious effect on health or egg production. However, the chloride content of the seaweeds caused considerable increases in water consumption and correspondingly watery droppings. Thus, with regard to the points where the two sets of experiments are comparable, they seem to be in very good agreement.

It may therefore be stated that 20% and 15% seaweed are too high levels for feeding, but that 10% appears to be safe, although it does not bestow any special advantage upon the ration.

It should, of course, be pointed out that our interest was chiefly in the mineral metabolism of the birds and that, under commercial conditions, these particular types of seaweed would probably not have been fed; and even if fed, they would not have been given in this way. The findings therefore refer specifically to the experimental conditions imposed.

Acknowledgments

This work forms part of the programme of research and development on seaweed undertaken by the Institute of Seaweed Research to whom the authors are indebted for permission to publish.

It is also a pleasure to thank Mr. T. Pearson for his technical assistance with the analyses and for his expert care and management of the birds.

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Received 28 April, 1955; amended manuscript 16 August, 1955

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MANUFACTURE OF ALGAL CHEMICALS. VII.*—The Isolation of Algal Carbohydrates by Means of Charcoal Columns

By W. A. P. BLACK, E. T. DEWAR and F. N. WOODWARD

Charcoal columns have been examined as a means of separating D-glucose and L-fucose obtained on acid hydrolysis of brown seaweeds. Glucose and fucose can be isolated on the one-gramme scale from synthetic mixtures and from hydrolysates of appropriate seaweeds on a column (46 × 5·0 cm.) with water as eluant; but difficulties are encountered on scaling up the process. Demineralization of mannitol–salt mixtures and the isolation of laminaribiose from partially hydrolysed laminarin can also be effected on charcoal columns.

Introduction

Previous methods described for the preparation of L-fucose from brown seaweeds possess certain disadvantages; its isolation from whole seaweed[1] involves the use of phenylhydrazine to give the relatively insoluble fucose phenylhydrazone, whilst that from hydrolysed fucoidin[2] necessitates the preparation of pure fucoidin which is a lengthy and difficult procedure.[3] Recently, Whelan & Morgan,[4] and Lindberg & Wickberg,[5] have separated a mixture of glucose methyl ethers on charcoal–Celite columns, using aqueous ethanol and ethyl methyl ketone as eluants. The possibility existed, therefore, that D-glucose and L-fucose, the two chief sugars obtained on hydrolysis of brown seaweeds, might also be separated on charcoal columns, in view of the different number of hydroxyl groups possessed by the two sugars.

A mixture of glucose and fucose can readily be separated by partition chromatography on a cellulose column,[6] using 90% *iso*propanol or *n*-butanol saturated with water as mobile phase, but this method is never likely to be applicable to large-scale separation.

The experiments described below include the isolation of both L-fucose and D-glucose, although it is realized that this method could never compete with established procedures for preparing glucose. Even for seaweed, it would be much simpler to prepare glucose by the hydrolysis of insoluble laminarin.[7]

* Part VI: *J. Sci. Fd Agric.*, 1953, **4**, 85.

The use of charcoal columns for demineralizing mannitol–salt mixtures and for preparing laminaribiose from laminarin has also been investigated.

Experimental

Species examined

The composition of the samples of the two species of seaweed used in these investigations is recorded in Table I.

Table I

Analysis of seaweed species examined

Species	% Chemical composition (dry basis)						
	Laminarin	Fucose (as $C_6H_{12}O_5$)	Mannitol	Ash	Alginic acid	Cellulose	Organic N
Fucus vesiculosus (Fv/XII/45/ME)	2·6	9·20	9·0	26·0	15·0	—	1·20
Laminaria cloustoni (C/X/48) frond	29·2	3·14	15·4	20·3	10·0	5·4	1·70

Unless otherwise stated, all rotations were measured in a 2-dm. tube, and all paper chromatograms were run in *n*-butanol–ethanol–water (40 : 11 : 19 by vol.) and sprayed with benzidine–trichloroacetic acid.[8]

Separation of glucose–fucose mixtures on charcoal–Celite

(1) *Using charcoal–Celite column* (46 × 5·0 *cm.*).—The column was prepared as described by Whelan, Bailey & Roberts.[8] To prevent the growth of moulds when not in constant use, the column was washed through with 0·1% mercuric chloride solution after each experiment. Organic solvents were avoided (except in the preparation of laminaribiose) because aqueous ethanol is difficult to remove even on prolonged washing with water[9] and it also alters the characteristics of the column.

Preliminary experiments showed that it was impossible to separate a mixture of glucose, fucose and mannitol (500 mg. of each) on this column, using water as the eluant. Glucose and mannitol emerged from the column almost at the same time. A mixture of glucose and fucose, however, has been successfully separated as described below.

D-Glucose (997 mg.) and L-fucose (986 mg.) were dissolved in water (20 ml.), the solution added to the column, and the column eluted with water under gentle suction at the rate of 200 ml./h. Fifty-ml. fractions were collected and the elution of the sugars was followed by measuring the optical rotation (α_D) of the fractions (Table II).

Table II

Optical rotation of the fractions of eluate

Fraction No.	1–18	19	20	21	22	23	24	25	26	27–34	35
α_D	0·00	0·08	0·32	0·85	0·66	0·14	− 0·19	− 0·49	− 0·62	N.D.	− 0·01

N.D. = not determined

Appropriate fractions were then combined as shown in Table III, concentrated to dryness at 45° under reduced pressure, dried with ethanol–benzene, weighed and the specific rotations determined. The composition of the syrups was calculated from the rotations, taking the equilibrium $[\alpha]_D$ of D-glucose as + 52·7° and that of L-fucose as − 76·0° in water.

These results show that more than 90% of each sugar has been separated in a high degree of purity. The facts that the total weight of syrup recovered (column 2) is slightly more than that of the starting material, and that both the glucose and fucose balances (columns 7 and 8) exceed 100%, can be attributed to the presence of a small amount of moisture in the dried fractions.

The rate of elution was found to be an important factor in obtaining pure fractions. When the above experiment was repeated with an elution rate of 500 ml./h., 82·0% of the glucose was

separated in 98·1% purity, but the fucose syrup was badly contaminated with glucose (0·927 g.; $[\alpha]_D^{19}$ − 54·8°, i.e., fucose, 83·5%; glucose, 16·5%), and this was confirmed by chromatographic analysis.

The above results show that glucose is eluted by a much smaller volume of water than is fucose, which tends to have a long 'tail'; but it is advisable not to introduce ethanol into the eluant for the reasons mentioned above.

Table III

Fraction No.	Wt. of dried syrup (mg.)	$[\alpha]_D^{16}$ in water	Concentration *c*	% Chemical composition		Glucose separated as % of total glucose	Fucose separated as % of total fucose
				Glucose	Fucose		
19–22	914	+ 52·0°	1·828	99·4	0·6	91·1	0·6
23	145	+ 23·8	0·725	77·6	22·4	11·3	3·3
24	79	− 46·2	0·395	23·1	76·9	1·8	6·2
25–35	917	− 72·5	1·834	2·7	97·3	2·5	90·5

(2) *Using charcoal–Celite column* (113 × 7·3 *cm.*).—Three experiments have been carried out with this larger column, which has approximately five times the volume of the previous one, to compare the efficiencies of the two columns. In experiment A, glucose (5·012 g.) and fucose (5·020 g.) were placed on the column and eluted with water at the same rate as in the small-scale experiment, viz. 200 ml./h. Fifty-ml. fractions were collected as before and the elution of the sugars was followed polarimetrically as shown in Fig. 1A. The rotation of the combined dextrorotatory fractions (112–151) showed a 99·9% recovery of D-glucose, indicating a complete separation of the two sugars. In experiment B, the quantity of each sugar was doubled but the elution rate was kept constant at 200 ml./h. Part of the elution curve is shown in Fig. 1B and the results are summarized in Table IV. Separation of 79·5% of the glucose in 97·3% purity was effected, but the fucose fraction was impure. The low recovery of fucose (78·6%) is due to the incomplete elution of this sugar from the column; fraction 225 still possessed α_D of − 0·07°. Very large volumes of water are required with this column to elute completely all the fucose present.

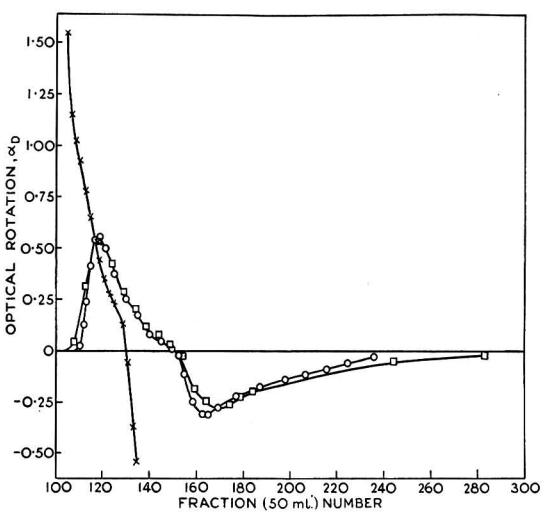

FIG. 1.—*Elution curves for experiments A, B and C*

O—O—O Experiment A
X—X—X Experiment B
□—□—□ Experiment C

Table IV

Composition of the syrups obtained in experiments B and C

Experiment No.	Wt. of glucose added (g.)	Wt. of fucose added (g.)	Rate of elution (ml./h.)	Total volume of combined fractions (ml.)	Wt. of syrup recovered (g.)	$[\alpha]_D$ in water	Concentration, *c*
B	10·00	9·910	200	1200	8·175	+ 49·3°	1·872
				200	0·339	+ 16·2	0·678
				4700	9·328	− 50·4	1·794
C	5·00	4·971	1000	2000	4·336	+ 52·0	1·752
				500	0·199	+ 6·0	0·994
				6450	4·364	− 61·3	1·345

Experiment No.	Fractions (50 ml.) combined	% Chemical composition		Glucose separated as % of total glucose	Fucose separated as % of total fucose
		Glucose	Fucose		
B	104–127	97·3	2·7	79·5	2·2
	128–131	71·6	28·4	2·4	1·0
	132–225	19·9	80·1	18·6	75·4
C	107–146	99·4	0·6	86·2	0·5
	147–156	63·7	36·3	2·5	1·5
	157–285	11·4	88·6	9·9	77·8

In experiment C, 5 g. of each sugar were eluted at 1000 ml./h., so that this experiment is comparable with the small-scale one described under (1). Because of the difficulty of removing traces of moisture from these syrups, concentrations of solutions after measurement of specific rotation were determined in this case by evaporating aliquot portions (containing about 200 mg. dry matter) and drying at 100° for 16 h. ; this treatment leads to a slight decomposition of the syrups, particularly where the fucose content is high, so that the resulting specific rotations may be slightly too high in this experiment. The results in Table IV show that 86·2% of the glucose can be isolated in the pure state, but the fucose is contaminated with glucose. It would appear, therefore, that this larger-scale experiment is not so efficient as that described under (1). Again, fucose was still being eluted from the column at fraction 285 (Fig. 1c).

Application of the method to hydrolysed Laminaria cloustoni *frond*

Treatment of a brown seaweed with N-sulphuric acid at 100° would be expected to hydrolyse the laminarin to D-glucose and the fucoidin to L-fucose, but to have little or no effect on the alginate or cellulose present, whilst the mannitol and salts would be brought into solution. After removal of the weed residue, therefore, the resulting hydrolysate should contain glucose, fucose, mannitol and salts, together with small quantities of other sugars, e.g., xylose, galactose and mannose,[10] which are also liberated on hydrolysis. Mannitol was eliminated by extracting the weed before hydrolysis with 85% methanol. It is necessary to use aqueous methanol, as anhydrous methanol fails to remove all the mannitol.

The method has been used to isolate glucose from *Laminaria cloustoni*. The dried, milled frond (3·116 g.) was refluxed three times with 85% (v/v) methanol (100 ml. each time) for 2 h., and then centrifuged and air-dried. It was hydrolysed with N-sulphuric acid (50 ml.) at 100° for 4 hr., the weed residue centrifuged, washed with water, the centrifugate and washings de-ionized with Zeo-Karb 225 and Amberlite IR-4B(OH), and the resulting solution concentrated *in vacuo* to dryness. The syrup (1·177 g.) was dissolved in water (20 ml.), the solution added to the column (46 × 5·0 cm.) and the column eluted with water (200 ml./h.) as previously described (Table V).

With the smaller quantity of fucose present (98 mg.), the separation is more clear-cut, fractions 33–35 (150 ml.) containing no sugars. Glucose does not emerge until later and the

glucose ' band ' is more diffuse (fractions 22–32). Fractions 23–30 were concentrated to a syrup (0·855 g.), $[\alpha]_D^{18}$ + 49·0° in water (*c*, 1·71), i.e., glucose, 92·9% (assuming impurities are inactive). Yield from the original frond, 78·5%.

Chromatography showed the complete absence of fucose in the syrup, although a trace of an unknown ($R_{glucose}$ 0·43) was detected. In view of the small quantity present (98 mg.), no attempt was made to isolate the fucose from the other fractions.

Table V

Optical rotation of eluate fractions (50 *ml.*)

Fraction No.	1–21	22	24	26	28	30
α_D	0·00	0·02	0·14	0·45	0·27	0·11
Fraction No.	32	33–35	36	38	40	42
α_D	0·02	0·00	− 0·01	− 0·02	− 0·03	− 0·02

Use of charcoal–Celite for removing salts from solution

Whelan *et al.*[8] found that sodium sulphate was eluted before glucose on a column, and suggested the use of charcoal for de-ionizing purposes. The technique has now been used for demineralizing mannitol–salt mixtures (see below), and has also been applied to seaweed hydrolysates. Dried, milled *Laminaria cloustoni* frond (3·132 g.) was extracted with 85% methanol and hydrolysed with N-sulphuric acid (50 ml.) as previously described. The hydrolysate was neutralized with 2N-sodium hydroxide, concentrated to 20 ml., and the solution fractionated on the column (46 × 5·0 cm.). Glucose was eluted in the same position as in the previous experiment (Table V). Fractions 8–22 (zero rotation) were evaporated to a mass of white salts (3·70 g. ; 50 ml. of N-Na_2SO_4 contain 3·55 g. of anhyd. Na_2SO_4). Fractions 23–30 yielded a colourless syrup (0·961 g.), $[\alpha]_D^{17}$ + 45·9° in water (*c*, 1·922), i.e., glucose, 87·1% (assuming impurities are inactive). Yield from the original frond, 82·4%.

The glucose syrup in this case is less pure than previously, but the purity is probably sufficiently high to allow crystallization from water on a larger scale. Chromatography (*tert.*-amyl alcohol–*n*-propanol–water 40 : 10 : 15 by vol.)[11] showed a trace of xylose in addition to glucose and the unknown, while the periodate–benzidine spray[11] also revealed the presence of mannitol, indicating that even 85% methanol does not remove all the mannitol from the frond.

Application of the method to Fucus vesiculosus

This alga contains xylose, 1·1%, and galactose, 0·4%, and a trace of mannose,[10] in addition to the constituents shown in Table I. It also yields, on hydrolysis, a considerable amount of a glassy material which does not move on a cellulose column[10] but which is eluted with water on a charcoal column in the same position as hexoses. Although it has not been examined in detail, this material is soluble in water, laevorotatory, insoluble in ethanol, and one sample gave ash, 12·1%; and $[\alpha]_D^{17}$ − 12° (water). *Laminaria cloustoni* frond did not give this material to anything like the same extent.

In spite of these difficulties, fucose has been isolated from *Fucus vesiculosus* in a fair degree of purity on the small scale. Dried, milled weed (10·05 g.) was refluxed three times with 85% methanol (120 ml. each time for 2 h.), hydrolysed with N-sulphuric acid (100 ml.) at 100° for 4 h., the hydrolysate and washings neutralized with 2N-sodium hydroxide, and concentrated *in vacuo* to 40 ml. After removal of a residue by filtering, the solution was added to the column (46 × 5·0 cm.), which was eluted with water (200 ml./h.). The elution of the sugars was followed polarimetrically (Table VI).

Table VI

Optical rotation of eluate fractions (50 *ml.*)

Fraction No.	1–22	23	25	27	29	31	33	35	41	48	50
α_D (4-dm. tube)	0·00°	− 0·06	− 0·26	− 0·12	− 0·06	− 0·14	− 0·75	− 0·73	− 0·30	− 0·06	− 0·03

The ' glassy material ' was responsible for the negative rotation up to fraction 30, after which fucose was eluted as evidenced by the much stronger laevorotation. Fractions 23–30 were combined to give a white glass (1·255 g. ; 12·5% of the alga), which was not further examined. Fractions 31–50 on concentration yielded a syrupy glass (1·381 g.), which was extracted with cold ethanol (2 × 25 ml.), and the extracts centrifuged to remove a white residue. The centrifugate was then concentrated to a syrup (0·889 g.), $[\alpha]_D^{18}$ — 67·5° in water (*c*, 1·778). Chromatography revealed the presence of fucose with traces of xylose, mannose, galactose and an unknown (R_F less than that of galactose). The syrup (0·860 g.), however, readily crystallized from ethanol (2 ml.) to give L-fucose (0·593 g.). Equilibrium $[\alpha]_D^{17}$ — 71·9° in water (*c*, 2·46), i.e., 94·5% L-fucose (assuming impurities are inactive) ; yield from the original seaweed, 62·7%.

The purity of the sugar compares favourably with that obtained by previous methods[1, 2] after one crystallization, while the yield is considerably improved.

However, when attempts were made to increase the quantity of fucose from 1 g. to about 5 g. on the same column, serious difficulties were encountered. Fucose began to emerge about ten fractions (500 ml.) in advance of its normal position, so that the separation of fucose from the other hexoses and xylose was less effective. This is illustrated by the following experiment:

The seaweed (50·55 g.) was extracted with 85% methanol, hydrolysed (500 ml. N-H_2SO_4), and the hydrolysate neutralized as described above. Since 500 ml. of N-sulphuric acid on neutralization give 35·5 g. of sodium sulphate, the bulk of this salt was precipitated with ethanol to avoid overloading the column with mineral matter ; in addition, ethanol removed a large proportion of ' glassy material '. The solution was, therefore, concentrated to 100 ml., ethanol (1 l.) added, the bulky precipitate centrifuged, and the centrifugate evaporated *in vacuo* to a brown mixture of sugars and salts (7·95 g.), which was then fractionated on the column (46 × 5·0 cm. ; 200 ml./h.). The following rotations were observed:

Table VII

Optical rotation of eluate fractions (50 *ml.*)

Fraction No.*	1–16	16½	17	17½	18	18½	19	19½
α_D	0·00°	0·00	— 0·02	— 0·13	— 0·17	+ 0·23	+ 0·48	— 0·35
Fraction No.*		20	20½	21	23	28	43	44
α_D		— 1·45	— 1·99	— 2·08	— 1·78	— 0·67	— 0·02	N.D.

* 16½ to 21 represent 25-ml. fractions

Fractions 17–18 contained ' glassy material ' and were rejected. Fractions 18½–19 yielded a colourless syrup (0·721 g.), chromatography of which showed xylose (strong), galactose/glucose (medium), an unknown (medium ; R_F less than that of galactose), mannose (trace) and fucose (trace). A solution of this syrup in water (14 ml.), when treated with ethanol (14 ml.), methylphenylhydrazine (1·4 ml.) and acetic acid (0·3 ml.) at 2° for 2 days, yielded galactose methylphenylhydrazone (0·139 g.) ; m.p. 183–184°, after recrystallization from ethanol ; mixed m.p. with D-galactose methylphenylhydrazone, 184–185°. This confirms the presence of galactose in *Fucus vesiculosus*.[10]

The fucose-containing fractions 20–44 were concentrated to a syrupy glass (5·55 g.), $[\alpha]_D^{17}$ — 56·3° in water (*c*, 2·22). This was extracted as before with cold ethanol (150 ml.), insoluble ' glassy material ' ($[\alpha]_D^{17}$ — 12° in water ; *c*, 0·925 ; ash, 12·1%) centrifuged off, and the centrifugate concentrated to a syrup (5·060 g.), the chromatography of which showed fucose (very strong), xylose (medium), galactose/glucose (weak), an unknown (trace ; R_F less than that of galactose) and mannose (trace). Dissolution of the syrup in ethanol (10 ml.) at 2° for 2 days gave the crystalline sugar (3·981 g.), which, however, was still impure. Equilibrium $[\alpha]_D^{16}$ — 63·0° in water (*c*, 2·222), i.e., 82·9% L-fucose (Found : ash, 1·0%).

These results show that the separation of the various sugars at this concentration is not sufficiently clear-cut to give a pure fucose fraction. With more than 1 g. of fucose in solution, the fucose ' band ' moves forward so that it becomes contaminated with other monosaccharides. It is doubtful, therefore, if this method could compete with previous methods[1, 2] for the large-scale preparation of fucose.

Demineralization of mannitol–salt mixtures

Mannitol–salt mixtures, obtained by aqueous acid extraction of seaweed samples followed by removal of laminarin and fucoidin by alcohol precipitation,[12] can be partially demineralized on charcoal–Celite, and this affords another method of isolating mannitol from these mixtures. The results in Table VIII were obtained with a mannitol (38·7%)–salt (37·9%) mixture (prepared from *Laminaria cloustoni* frond as previously described[12]) on the column (46 × 5·0 cm.). In both cases the mixture was dissolved in water (40 ml.), the solution added to the column and eluted with water at 200 ml./h., mannitol being detected by spotting on filter paper and spraying with periodate–benzidine reagent.[11] The fractions containing mannitol were combined to give

Table VIII

Demineralization of mannitol (38·7%)*-salt* (37·9%) *mixture*

Expt. No.	Wt. of mixture taken (g.)	Mannitol-containing fractions (50 ml.)	Wt. of crude mannitol (g.)	Analysis of crude mannitol		
				Ash %	Mannitol %	Mannitol as % of mannitol in mixture
1	14·64	20–31	6·75	10·5	84·6	100·8
2	29·93	18–19	5·787	38·3	38·4	19·2
		20–27	12·10	18·2	74·3	77·6

crude mannitol as a white crystalline solid. In experiment 1, the salt-containing fractions (8–19) were evaporated to yield a white mass of salts (5·60 g.), which gave an ash content of 91·0%, i.e., 91·9% of total ash in the original mixture.

The results show that the mineral content of mannitol–salt mixtures can be considerably reduced in this way, and pure mannitol can readily be obtained from the crude product by recrystallization from water. In experiment 2, the large amount of salt present (11·34 g.) rendered the separation less clear-cut, and the crude mannitol still possessed a high ash content (18·2%).

It has been found, however, that pure mannitol can be prepared from mannitol–salt mixtures, containing more or less any ratio of mannitol to salt, by direct crystallization from water, thereby eliminating the use of charcoal columns or any other method of separation.[12] This method is based on a Japanese patent.[13] The mannitol–salt mixture was extracted at 90° with water equal in weight to the mannitol present in the mixture, the undissolved salts filtered off from the hot solution, and the filtrate allowed to crystallize. The above mannitol (38·7%)–salt (37·9%) mixture (25·35 g.) was heated with water (10 ml.) at 90° and the salts filtered off, when the filtrate crystallized on cooling. After 16 h. at 2°, the crude mannitol was filtered, washed with saturated mannitol solution (5 ml.), ethanol and ether, and dried at 100° (6·049 g.). Found: ash, 10·1; mannitol, 90·9%. Yield, 56·0%. This crude material (5·628 g.), on recrystallization from water (5 ml.), gave pure mannitol (4·406 g.). Found: ash, 0·15%; m.p. 165–167°. Yield from the original mixture, 48·2%.

In both the direct crystallization method and the charcoal–Celite column, it is essential that the laminarin and fucoidin are first removed from the aqueous extract of the weed by alcohol precipitation. The presence of polysaccharides in the direct crystallization method makes the extract of the mannitol–salt mixture so viscous that it is impossible to remove the undissolved salts by filtration, while with charcoal–Celite, laminarin and fucoidin contaminate the column and are not readily eliminated afterwards. It has been found unnecessary in the preparation of mannitol–salt mixtures to raise the concentration of alcohol to 85% (w/w) to remove polysaccharides as previously described.[12] The dilute aqueous acid extract of the seaweed is neutralized, concentrated under reduced pressure to a viscous solution containing 10–15% (w/v) of mannitol, and ethanol or methanol added to 80% (v/v). The precipitated material, containing laminarin, fucoidin and some of the salts, e.g., sulphates, is then centrifuged, and the centrifugate evaporated to give the mannitol–salt mixture as a light-green or brown solid.

Preparation of laminaribiose from laminarin

The disaccharide, laminaribiose, was first isolated by Barry,[14] who heated laminarin with N-oxalic acid until the hydrolysis was about two-thirds complete (7 h., 100°), and then destroyed

the glucose with yeast. The optically active liquid remaining was dissolved in methanol, and the laminaribiose was obtained as a powder by fractional precipitation with ethanol and ether. Connell, Hirst & Percival[15] also obtained the disaccharide by partial hydrolysis of laminarin with oxalic acid and separation of the sugars on a cellulose column. Proof of the structure of laminaribiose as 3-*O*-*β*-D-glucopyranosyl-D-glucose has been obtained by its synthesis from 1 : 2-5 : 6-di-*O*-*iso*propylidene-glucose and tetra-*O*-acetylglucosyl bromide.[16, 17] Peat, Whelan & Lawley[18] used charcoal–Celite columns for separating laminaribiose, gentiobiose, *ββ*-trehalose and five trisaccharides from a partial hydrolysate of insoluble laminarin. Recently, they have shown that the material thought to be *ββ*-trehalose is, in fact, 1-*β*-glucosyl-mannitol.[19]

Laminaribiose can readily be prepared from laminarin by the use of charcoal–Celite columns. Laminarin (25·02 g.; from *Laminaria cloustoni* frond) was partially hydrolysed with 0·25N-sulphuric acid at 100° for 5 h. (% hydrolysis, 52·2);[7] the hydrolysate was centrifuged to remove a small residue, and the solution was neutralized with Amberlite resin IR-4B(OH) and concentrated at 45° under reduced pressure. The resulting syrup (28·20 g.) was dissolved in water (40 ml.), the solution added to the column (46 × 5·0 cm.) and the column eluted as described by Whelan *et al.*[8] in their preparation of the maltodextrins. Fractions of 150 ml. were collected and the elution of the sugars with water and 7·5% ethanol was followed polarimetrically as shown in Fig. 2. The rotation of the combined dextrorotatory fractions (7–11) obtained with water as eluant showed 13·2 g. (47·5% yield from laminarin) of glucose present (13·5 g. by hypoiodite estimation[20]).

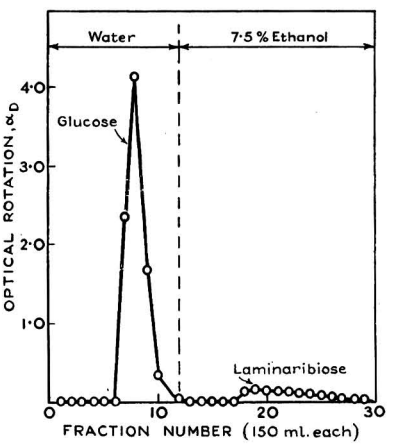

FIG. 2.—*Chromatographic separation of glucose and laminaribiose on charcoal*

The disaccharide fractions (18–30) obtained with 7·5% ethanol were evaporated to a glass (5·10 g.), $[\alpha]_D^{12} + 19{\cdot}9°$ in water (*c*, 1·205). Chromatography (*n*-butanol–pyridine–water–benzene 5 : 3 : 3 : 1 by vol.) showed laminaribiose ($R_{glucose}$ 0·69) and an unknown ($R_{glucose}$ 0·29), but glucose was completely absent. The disaccharide was crystallized by dissolving the glass (4·40 g.) in anhydrous methanol (30 ml.) and adding ethanol (30 ml.), when crystals formed almost immediately. After 3 h. at 2°, the first crop (0·189 g.) was filtered off and rejected. The filtrate, after 7 days at 2°, gave crystalline laminaribiose, which was filtered, washed with ethanol (5 ml.) and dried *in vacuo* over phosphorus pentoxide to a white powder (3·234 g.). Yield, 14·2% (from laminarin); $[\alpha]_D^{20} + 22{\cdot}4°$ (4 min.) → + 17·8° in water (*c*, 2·0); Bächli & Percival[17] quote $[\alpha]_D^{16} + 24{\cdot}9°$ (20 min.) → + 18·6° in water for synthetic α-laminaribiose; m.p. 198–201° (uncorr.). Found: ash, ± 0·0; C, 41·9; H, 6·6. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·5%. Estimations by hypoiodite oxidation,[20] however, were invariably low (92·0 — 92·4%) for this disaccharide. Chromatography (*n*-butanol–pyridine–water–benzene 5 : 3 : 3 : 1) showed a single spot.

Summary

(1) A charcoal–Celite column (46 × 5·0 cm.) can effectively separate a mixture of 1 g. of glucose and 1 g. of fucose, using only water as the eluant; more than 90% of each sugar was recovered in 97–99% purity.

A column (113 × 7·3 cm.) of approximately five times the volume will separate a mixture of 5 g. of glucose and 5 g. of fucose, when the rate of elution with water (200 ml./h.) is kept the same as on the small scale. When the elution rate is increased five times (1000 ml./h.), the fucose becomes contaminated with glucose.

(2) D-Glucose has been isolated on the 1-g. scale, in 78% yield and 93% purity, from hydrolysed *Laminaria cloustoni* frond, from which mannitol had previously been eliminated by 85%-methanol extraction. The separation from fucose was complete, although the sugar was slightly contaminated with traces of unidentified material.

When the column was used both as a means of separating the sugars and for demineralization, glucose was isolated in 82% yield and 87% purity.

(3) L-Fucose has been isolated on the 1-g. scale, in 63% yield and 94% purity, from methanol-extracted, hydrolysed *Fucus vesiculosus*. This compares favourably with previous methods for obtaining fucose from seaweeds.

Hydrolysates of *Fucus vesiculosus* contain, however, in addition to fucose and glucose, various other constituents, e.g., xylose, galactose and a laevorotatory, unidentified 'glassy material', which interfere with the separation of fucose on charcoal–Celite columns. When the experiment was scaled up to the 5-g. fucose level on the same column (46 × 5·0 cm.), it was not possible to separate a pure fucose fraction. It is difficult to see how the method could compete with previous methods for the large-scale preparation of fucose.

(4) The presence of D-galactose in hydrolysates of *Fucus vesiculosus* has been confirmed by the isolation of its methylphenylhydrazone.

(5) The demineralization of mannitol–salt mixtures on charcoal–Celite has also been investigated and compared with the direct crystallization of mannitol from concentrated aqueous mannitol–salt solutions. The capacity of the column is again the limiting factor, and it would appear that direct crystallization is the simpler method. A mannitol (38·7%)–salt (37·9%) mixture, after two crystallizations from water, gave pure mannitol in 48% yield.

(6) Crystalline laminaribiose has been prepared in 14% yield by the partial hydrolysis of laminarin and chromatography of the hydrolysate on charcoal. Glucose was completely eliminated with water, and the disaccharide was obtained by elution with 7·5% ethanol. The sugar readily crystallized with a 1 : 1-methanol–ethanol mixture.

Acknowledgments

The work described in this paper forms part of the programme of research and development on seaweed undertaken by the Institute of Seaweed Research, and the authors are indebted to the Institute for permission to publish. They are also indebted to Dr. W. J. Whelan, University College of North Wales, Bangor, for help and advice on the use of charcoal columns, particularly with the early work on pure glucose–fucose mixtures.

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Received 14 April, 1955

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SOME OBSERVATIONS ON THE 'RESEARCH' EXTENSOMETER*

By I. HLYNKA

A comparative study of the 'Research' Extensometer and the Brabender Extensograph has been made using three different schedules for the test samples and three types of dough holders with the Extensometer.

In general, differences in the procedure of handling dough-pieces cause more effect on the shape of the load–extension curve than do differences between machines. Impaling of the dough directly on the Extensometer modifies the shape of the load–extension curves: this is attributed to structural activation of the dough in the impaling process. An Extensometer dough holder is described which permits the dough to be rested between impaling and stretching.

For structural relaxation studies the range of extensibility provided by the Extensometer must be increased by lengthening the drive shaft.

Introduction

In 1949 Halton[1] described a new instrument for obtaining load–extension curves on dough. This instrument is now manufactured under the name of 'Research' Extensometer and is widely used in the United Kingdom. The Extensometer was designed for testing fermented doughs in parallel experiments corresponding to various stages in the baking test. In addition it may be used for unleavened doughs and is, in principle, essentially the same as the Brabender Extensograph. It was puzzling, however, that the load–extension curves obtained with the two instruments appeared to be quite different in shape.

In this laboratory the Brabender Extensograph has been used extensively[2, 3, 4] in studies of dough rheology. Since the Extensometer appears to offer some advantages, a short comparative study of it and the Extensograph has been carried out. The results are summarized in this report.

Experimental

Materials and methods

Straight grade flour of 13·1% protein, milled to about 72% extraction on a laboratory Buhler mill from Canadian spring wheat grading No. 2 Northern, was used in all experiments. All doughs were unleavened and were made up with 1% salt to an absorption of 63·2%. All data are referred to flour containing 14% moisture. The doughs were mixed in a GRL laboratory dough mixer[5] for 3 min. and 100 g. of dough for each test were used on both instruments.

* Paper No. 144 of the Grain Research Laboratory, Board of Grain Commissioners for Canada

The two instruments were compared with three different test procedures, and with three different holders with the Extensometer only; the Extensograph served as a reference for comparison.

The three procedures of handling dough were as follows:

(*a*) The Extensometer procedure for unleavened doughs, as outlined in the manufacturer's book of instructions, was followed; i.e., mix dough, scale off a 100-g. sample, relax for 1 hour, mould, relax for 5 min., impale and stretch.

(*b*) The usual Extensograph procedure was used, i.e., mix, scale off sample, mould (round and shape), place on holder, relax 45 min. and stretch.

(*c*) Lastly, the Extensograph procedure for doughs containing bromate was included with the following steps: mix, scale off sample, allow reaction time of 135 min., mould, place on holder, relax for 45 min. and stretch. These are subsequently called Extensograph procedures I and II. In all tests, samples of dough were rounded 20 times in the Extensograph rounder in order to give the doughs a uniform treatment.

The three dough holders used with the 'Research' Extensometer were as follows:

(*a*) The sample was impaled directly on the dough pins of the Extensometer just prior to stretching.

(*b*) A dough holder from the Relaxometer[6, 7] was adapted for use on the Extensometer. This holder made it possible to impale the dough on the holder at the time of moulding, to allow a relaxation period, and then to place the sample on the Extensometer without disturbing it.

(*c*) A regular Brabender Extensograph dough holder was adapted for use on the Extensometer.

Figs. 1–3 illustrate the details of the adapted dough holders. Fig. 1 shows the Relaxometer dough holder (E, F) and accessories (A to D) used for a more uniform mechanical loading of the dough. To load, the holder is first assembled by placing the cap E on the two halves of the holder. Then the piston A is inserted from the right into the cylinder B and is followed by the dough sample which is pressed firmly into position by the packer C. The cylinder with the dough is then placed in position in the dough holder and the dough is impaled by pushing down the piston A. Next, the cutter D is inserted into the shaft of piston A to engage the cap E of the holder; it is turned to cut off a small piece of dough which finds its way into this position. The cutter D is then used to hold down the dough holder while the cylinder B is pushed up and is disengaged from the dough. Finally, the flat surface of the piston is disengaged from the dough

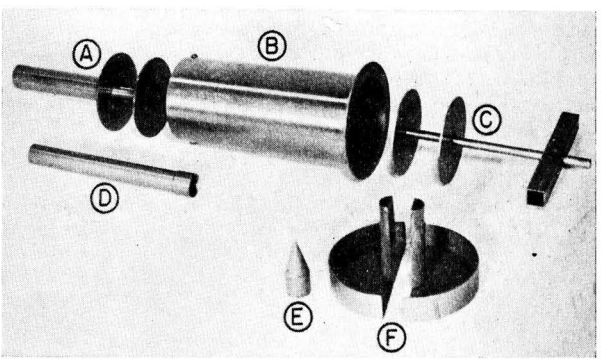

FIG. 1.—*Relaxometer dough holder and accessories*

with the aid of a spatula. The formed dough may now be allowed to rest undisturbed in a humidity cabinet until required. For stretching, the holder is quickly placed on the Extensometer pins, the cap is removed, and the machine started.

Fig. 2 shows the parts of a Brabender dough holder which is designed to hold a cylindrical test piece of dough clamped at either end. To prevent sagging of the central portion of the dough

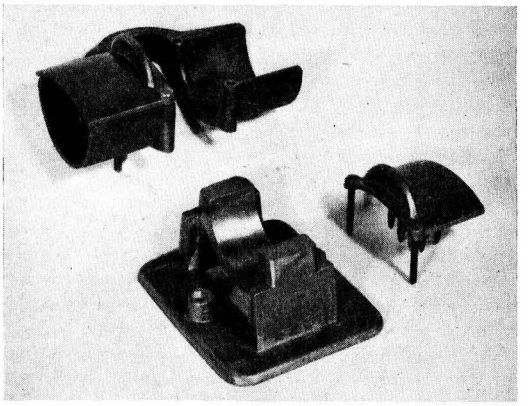

FIG. 2.—*Brabender dough holder*

cylinder, the holder is kept on the cradle shown in the foreground. Fig. 3 shows the Extensometer with the pins replaced by a carriage to accommodate the Brabender dough holder, and a hook to extend the dough. These are, of course, improvisations modelled on the design of the Extensograph.

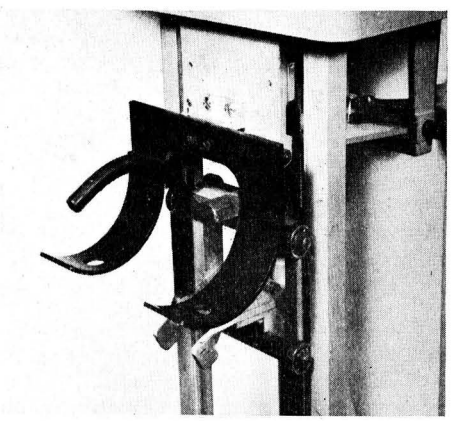

FIG. 3.—*Extensometer equipped with a carriage to accommodate Brabender dough holder*

Results

Fig. 4 summarizes the comparison of the Extensometer with the Extensograph in the form of load–extension curves. The top row of curves was obtained with the Extensograph using the three procedures of dough handling already outlined, and simply referred to here as Extensometer, Extensograph method I and II. The high curve with the Extensometer method results from the relatively short rest period of 5 min. allowed between moulding, and stretching the dough. The curve with the Extensograph II method is also high because the dough in this test contained 40 p.p.m. of bromate. In order to make a direct comparison of the curves obtained with the two instruments, this top row of Extensograph curves is repeated as dotted curves in all the succeeding sets of curves shown in Fig. 4.

The second row of curves shows the results obtained with the Extensometer. The doughs were impaled by hand in each of the three schedules of dough handling. Because it was desired to compare the shape of the curves rather than the dimensions (which are given in a subsequent table) the actual Extensometer curves were recalculated to the same length as the corresponding Extensograms, and to the same height at the midpoint. This device serves to bring out the main differences between the curves produced by the two machines. All the impaled doughs gave curves that rise very much more rapidly to a maximum and fall off more gradually in comparison with Extensograms.

A third set of Extensometer curves was obtained under the same conditions except that the dough was impaled on the Relaxometer holder at the time of moulding the test piece. Thus once the dough was placed on the holder it was not disturbed further until it was stretched on the instrument. The difference between Extensometer and Extensograph curves was similar in kind to that obtained for impaled doughs, but the magnitude of the difference was greatly reduced especially with procedures which permitted a long rest period.

The curves shown in the bottom row of Fig. 4 were obtained using an Extensograph holder on the Extensometer. The results show that the curves obtained on either instrument but using the same dough holder are practically identical in shape.

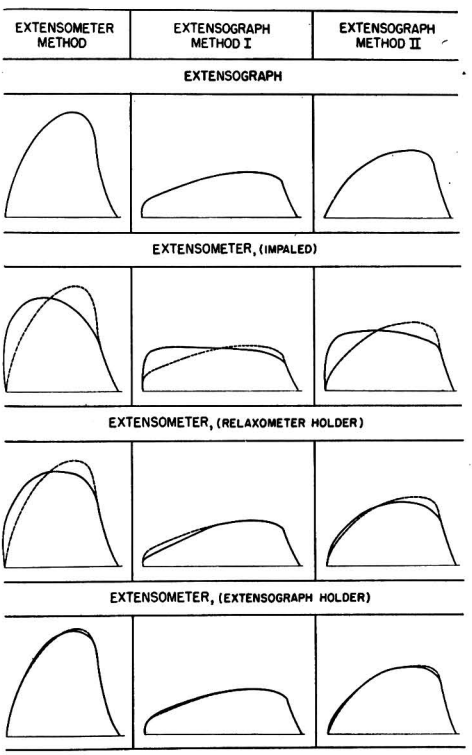

FIG. 4.—*Comparison of load–extension curves produced by 'Research' Extensometer and by Brabender Extensograph using three schedules for preparing test samples, and three dough holders with the Extensometer*

The foregoing results are further amplified in Table I which presents data on the extensibilities of the curves shown in Fig. 4. The results are expressed as distance of dough hook travel in cm. It should be pointed out that the maximum length of travel possible in the Extensometer is 60·4 cm. This is inadequate for doughs allowed longer relaxation times. Accordingly, a

longer drive shaft was installed to increase the total travel of dough hook to 90 cm. With the added length the kymograph drum was allowed to rotate more than once in a manner used with the Relaxometer.[7]

Table I

Comparative dough extensibilities with varying procedures

Instrument	Dough holder	Procedure		
		Extensometer cm.	Extensograph I cm.	Extensograph II cm.
Extensograph	own	28·5	41·0	34·0
Extensometer	impaled	37·0	50·3	30·2
Extensometer	Relaxometer	48·8	80·0	52·6
Extensometer	Extensograph	30·6	46·6	37·0

The data in the first and last line show that the more rapid extension on the Extensometer (2·09 cm./sec. compared with 1·46 cm./sec. on the Extensograph) gives a greater extensibility to the dough with the same holder. The Relaxometer holder on the Extensometer gives the greatest extensibility. Impaling the dough by hand definitely shortens the extensibility.

The difference between the Extensograph and the Relaxometer holders should be noted. The Extensograph holder does not utilize the entire piece of dough. The ends of the test piece that are pinned through are immobilized, and only the central portion is used. In the Relaxometer holder and in doughs impaled directly on the Extensometer pins, the entire dough is used and an adjustment around the pins takes place as the dough flows into the actively stretching strand. This, as well as the increased rate of extension, results in greater extensibility. Another feature of the Extensograph holder is that, during stretching, the dough flows against three surfaces (two on the holder and one on the hook); two of these are on the outside and one on the inside of the dough strand. In the Relaxometer holder the dough is stretched against two surfaces, both on the inside. This determines the type of surface and the type of necking on the dough strand. In general, the Extensograph holder appears to give better reproducibility of results.

Discussion

Impaling test samples of dough manually on the Extensometer changes the shape of the load–extension curves, and decreases the extensibility as well. In the terminology used in this laboratory, part of the test piece is 'structurally activated'[2, 3] by the impaling process. The load–extension curve thus reflects not only the properties of the dough, but also the effect of the impaling process. This procedure therefore introduces an unnecessary complication in the dough test piece. For rheological studies, a procedure giving an uncomplicated load–extension curve is preferred.

There is a small difference between the curves in which a split-pin dough holder was used, and the curves obtained with an Extensograph holder. The difference may be attributed to the design of the holder itself and the manner in which dough flows during the extension process. However, provided that the curves obtained with any one holder belong to the same family of curves, evaluation of data such as load at constant sample extension is valid on the basis of similarity analysis.

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Received 25 February, 1955

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SOIL CONTAMINATION OF HERBAGE SAMPLES

By A. THOMPSON and A. M. RAVEN

Soil-free plant material has been artificially contaminated with soil to definite levels and the resulting samples analysed for the commonly determined major and trace mineral elements. Considerable errors resulted from such analyses.

Various degrees of washing of plant material prior to analysis have been examined to find the leaching losses that such treatments may cause. All but a spray method were found to cause considerable loss of both major and trace elements.

Several known methods of estimating the degree of soil contamination in herbage samples have been compared, and a procedure based upon the determination of aluminium is outlined.

Introduction

In recent years, the nutritional role of the inorganic elements has been the subject of much research. This has resulted in the publication of a considerable literature concerning the mineral composition of herbage and other crop plants. The accumulation of such valuable information has been facilitated by the advances made in analytical techniques during the past thirty years. Relatively crude gravimetric procedures have given way to a whole range of colorimetric, spectrographic and polarographic methods. In competent hands, it is reasonable to assume that such methods can attain an accuracy consistent with the requirements for this type of work. Thus methods are available for the commonly determined elements, with the possible exception of cobalt, which allow of an accuracy greater than $\pm 5\%$. It may justifiably be concluded that, once a sample is prepared for analysis, major errors are unlikely to occur in the subsequent determination.

That the value obtained is, however, a true reflection of the composition of the material growing in the field is by no means so certain. Contamination of the sample by soil, and that arising during its preparation for analysis, may in fact have contributed quite considerably to the final result. In some published mineral studies, brief reference is made to precautions taken to ensure freedom from contamination. This is at least an acknowledgment of the problem, though on many occasions it is doubtful whether the precautions reported are either sufficient or effective.

Whilst it is true that many writers, notably Askew & Dixon[1] and Russell,[2] have referred to soil contamination as a possible cause of certain abnormal results, very few have given the matter the attention it appears to warrant. There have, however, been a few isolated attempts to assess the probable extent of such contamination, such as those of Woodman *et al.*,[3] Askew & Rigg[4] and Shorland.[5] Their results suggest that levels up to 5% of extraneous mineral material in the dry matter of herbage samples are by no means uncommon. Existing analyses of soils and plants such as those of Browne[6] and Mitchell[7] indicate that such levels of contamination would probably cause very considerable errors in the determination of the mineral composition of herbage, notably in respect of the trace elements.

In view of the paucity of factual information concerning soil contamination in the mineral analysis of plant materials, the present study attempts, firstly, to estimate the probable effects of such contamination upon analytical results and, subsequently, to consider possible methods of minimizing such effects.

Experimental

The effect of degree of contamination upon analytical results

Previous investigations into soil contamination appear to have been hindered by the difficulty of obtaining herbage which could be regarded as being free from extraneous soil. In the work under discussion such material was grown by means of soil-less culture using thoroughly washed gravel as the supporting medium. This was contained in galvanized iron tanks, the inside of which had been coated with non-toxic bituminous paint. The nutrient solution was stored in a similarly treated tank situated beneath the gravel bed.

Commercial fertilizers were used in compounding the nutrient solution, which supplied approximately 400 p.p.m. of potassium, 200 to 300 p.p.m. of both nitrogen and calcium, 50 to

60 p.p.m. of phosphorus and of magnesium, along with certain trace element additions. A sub-irrigation pumping system was adopted as being the most suitable for the purpose, and was controlled by a time-clock mechanism. The latter was set so that the pump operated for a sufficient time to saturate the gravel bed three times daily. As most of the plant material had to be produced during the winter months, artificial heating and lighting were necessary. Seeds of Italian ryegrass were sown directly on to the gravel surface, covered, and irrigated with water until germination had occurred. After reaching a height of 6 to 8 inches in approximately 3 to 4 weeks, the herbage was cut using a pair of nickel–chromium shears,* to avoid the possibility of iron contamination.

Immediately after cutting, the soil-free material was dried on paper-lined trays, in a Birmingham Blackburn Drier at 60° for 24 hours, milled in a chromium-plated Christie and Norris mill using a $\frac{1}{32}$-inch sieve, and bottled ready for analysis.

A sample of soil from the Nursery of the King's College Experimental Station at Cockle Park was collected, dried and finely ground. This particular soil was chosen for use in the present study as a considerable volume of analytical data was available for plants grown in it.

The necessary amounts of dry soil and plant material to produce samples of certain definite soil contents were calculated, and a series containing 0, 2, 4, 6, 8 and 10% of extraneous soil was prepared. To ensure uniform contamination between replicate samples, the two constituents were weighed separately for each determination and then mixed.

The artificially contaminated samples were analysed for total ash and silica by the conventional procedures, calcium by the modified McCrudden method as quoted by Piper,[8] magnesium as recommended by the A.O.A.C.,[9] phosphorus by a slightly modified Richards & Godden procedure,[10] potassium using the procedure of Krügel & Retter,[11] sodium by the method of Kahane,[12] and chlorine by that of Husband & Godden[13] modified by Caldwell & Moyer.[14] Of the trace elements the following were determined: iron using the method of Parker & Griffin,[15] manganese by that of Richards,[16] copper as recommended by Forster,[17] and cobalt by the procedure of Stare & Elvehjem.[18]

The results shown in Table I indicate that the silica-free ash, calcium and sodium determinations are little affected by contamination with up to 10% of soil. The reason for this may be seen in the great similarity between the levels of these constituents, in both soil and plant. The authors feel, however, that it would be unwise to generalize on this point as certain calcareous soils, for example, may exert considerably greater effects than have occurred in the present instance.

In the determination of the remaining major elements, excluding silica and total ash, the percentage error incurred by the inclusion of 10% of soil is seen to be negative, and of the order of 9%. The apparent decrease of these elements in the samples caused by increasing contamination appears to be merely one of dilution, due to the lower levels of potassium, chlorine, magnesium and phosphorus in the soil. It should, perhaps, be pointed out that the use of a nutrient solution rich in potassium and phosphorus has resulted in high levels of these two elements in the original plant material. Under more normal cultural conditions the error caused by contamination would in all probability be less than has occurred in the present case.

The levels of the remaining mineral constituents are all seen to rise with increasing soil contamination, and only in the case of copper is the correlation coefficient not significant. However, as the soil contains only a slightly greater amount of this element than does the uncontaminated herbage, the negligible effect of soil contamination upon the copper contents of the samples is not unexpected. The total ash, silica, iron, manganese and cobalt determinations show very considerable errors due to the extraneous mineral matter, caused by the very high contents of these elements in the soil as compared with the herbage. The errors are of such magnitude that even small soil contaminations cannot be ignored if accurate results are to be obtained. Thus the inclusion of as little as 1% of soil in a herbage sample may increase the iron content by nearly 100% of its true value.

In view of the considerable errors caused by soil contamination in the mineral analysis of herbage, Mitchell[19] has suggested that a more reliable assessment of the mineral status of pastures

* The authors wish to acknowledge the assistance given by the Wilkinson Sword Co. in constructing these shears.

might be obtained by an analysis of the underlying soils, rather than of the herbage itself. However, in view of the accumulating evidence that the ash composition of plants is governed by several factors other than soil conditions, the above suggestion is of limited value. The distribution of several species of plants in a sward, each species often showing characteristic mineral composition, the maturity of the herbage and other such factors, combine to render soil data somewhat unreliable in assessing the mineral potential of a particular sward. It would appear, therefore, that despite the possibility of contamination of samples, plant analysis still remains the most reliable method of assessing the value of pasture as a source of minerals for livestock.

The removal of contaminating material by washing

The most direct approach to the problem of minimizing contamination errors, would appear to be that of removing the extraneous mineral matter by washing the herbage. Such a procedure has been rejected by many workers,[5, 8, 20, 21] on the grounds that excessive leaching of the inorganic constituents occurs. However, uncertainty exists as to the degree of leaching which might be caused by a particular washing treatment. This is partly due to the fact that previous workers appear to have gone to excessive lengths, even to shaking for 24 hours with water, to prove that leaching does occur.

In the present investigation it was thought desirable to establish the degree of leaching which might be expected by washing treatments of varying duration. To this end, Italian ryegrass, grown in the manner as previously described, and free from soil, was divided into four equal portions immediately after cutting, each portion being subjected to one of the following four treatments:

(*a*) Control;

(*b*) 50 g. of the wet material were spread out on an aluminium tray and sprayed with glass-distilled water from a power spray for 3 minutes. The spray was very fine and the total volume of water used was 400 ml.;

(*c*) 50 g. of the sample were soaked in 2 litres of glass-distilled water and were continuously agitated for 5 minutes. At the end of this time the water was decanted off from the grass;

(*d*) 50 g. of the ryegrass were soaked in 2 litres of glass-distilled water for 1 hour. The suspension was agitated for 1 minute immediately after adding the water, and thereafter for 1 minute every 15 minutes. At the end of the 1-hour period the water was poured away, leaving the grass.

After treatment, all four portions were dried on paper-lined aluminium trays for 48 hours at 60°, milled and bottled as previously described. Each dried portion of the ryegrass was analysed for total ash, silica-free ash and the eleven elements previously mentioned, with the addition of zinc. The results of these analyses are shown in Table II.

It will be seen from the results shown in Table II that spraying with water for 3 minutes has little effect upon the analysis of the ash constituents of the ryegrass. In most cases variations caused by the spraying treatment are within the limits of experimental error. One notable exception was silica, which shows a loss of 12·5%.

The losses due to leaching become more appreciable with the 5-minute soaking treatment. Approximately one-third of the silica and cobalt are removed, while the manganese and chlorine losses exceed 10%.

Most constituents suffer very considerable losses as a result of soaking in water for an hour. Most of the major elements show decreases of the order of 10 to 20%, whilst the trace elements show losses ranging from less than 4·0% in the case of iron and zinc to 76·0% in the case of cobalt. In general, the above losses are in agreement with those found by Shorland.[5]

It will be obvious from the foregoing results that any prolonged washing treatment results in losses of such magnitude as to nullify accuracy gained by attempting to remove soil contamination in this manner. It would, indeed, be difficult to justify even a 5-minute soaking treatment, even assuming that this did in fact remove adhering soil. One possible exception to the above statement might be made. As the loss of iron, even after prolonged treatment with water, is small, the preliminary washing of herbage prior to the determination of this element would seem to be a relatively safe procedure. The negligible leaching caused by the spraying treatment,

Table I

The mineral composition of artificially contaminated samples of herbage. (Results expressed on a dry-matter basis)

Soil contamination	Total ash	Silica	Silica-free ash	Calcium (Ca)	Phosphorus (P)	Potassium (K)	Magnesium (Mg)	Chlorine (Cl)	Sodium (Na)	Iron (Fe)	Manganese (Mn)	Copper (Cu)	Cobalt (Co)
%	%	%	%	%	%	%	%	%	%	p.p.m.	p.p.m.	p.p.m.	p.p.m.
0	17·46	1·16	16·30	0·81	0·94	7·03	0·40	0·73	0·27	266	81	28·1	0·08
2	18·84	2·84	16·30	—	—	—	—	—	—	664	101	30·3	0·18
4	20·85	4·65	16·20	—	—	—	—	—	—	985	116	28·8	0·25
6	22·18	6·01	16·17	0·79	0·90	6·56	0·38	0·71	0·26	1381	127	28·8	0·35
8	23·35	7·88	15·47	—	—	—	—	—	—	1640	144	30·2	0·40
10	25·04	9·34	15·70	0·79	0·87	6·06	0·37	0·68	0·26	2113	155	30·1	0·45
Soil	94·48	83·60	10·88	0·51	0·27	0·43	0·03	0·20	0·21	18,740	825	35·0	4·00
% Error due to 10% soil	+43·4	+705·2	−3·7	−2·5	−7·5	−13·8	−7·5	−6·9	−3·7	+694·4	+91·4	+7·1	+462·5
Correlation coefficient	+0·992**	+0·999**	−0·762	−0·918	−0·999*	−0·991	−0·999*	−0·973	−0·915	+0·998**	+ 0·996**	+0·453	+ 0·992**
Regression coefficient	+0·743	+0·820	−0·066	−0·000	−0·007	−0·096	−0·003	−0·005	−0·001	+179·4	+ 7·286	+0·113	+ 0·037

* Denotes significance at 5% probability
** Denotes significance at 0·1% probability

Table II

The ash constituents of samples of ryegrass subjected to various washing treatments. (Results expressed on a dry-matter basis)

Treatment	Total ash	Silica	Silica-free ash	Calcium (Ca)	Phosphorus (P)	Potassium (K)	Magnesium (Mg)	Chlorine (Cl)	Sodium (Na)	Iron (Fe)	Manganese (Mn)	Copper (Cu)	Cobalt (Co)	Zinc (Zn)
	%	%	%	%	%	%	%	%	%	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Control unwashed	19·06	1·92	17·14	0·90	0·94	6·86	0·32	0·74	0·46	286	104	21·9	0·21	63·3
3-minute spray	18·71	1·68	17·03	0·92	0·95	6·86	0·33	0·74	0·44	287	105	22·0	0·21	63·5
Loss due to spraying, % of total	1·84	12·50	0·64	—	—	0·00	—	0·00	4·55	—	—	—	0·00	—
5-minute soak	18·31	1·25	17·06	0·87	0·97	6·40	0·33	0·66	0·44	290	89	20·3	0·14	63·6
Loss due to 5-minute soak, % of total	3·93	35·15	0·47	3·33	—	6·71	—	10·81	4·55	—	14·42	7·31	33·33	—
1-hour soak	17·72	1·43	16·29	0·81	0·99	6·16	0·27	0·58	0·37	279	90	20·2	0·05	61·0
Loss due to 1-hour soak, % of total	7·03	25·71	4·96	10·00	—	10·20	15·63	21·62	20·62	2·45	13·46	7·76	76·20	3·63

whilst of interest, does not allow of any definite recommendation. Further work is indicated to establish the maximum permissible level of spraying and the efficiency of such a treatment in removing the contaminating soil.

Indirect methods for the assessment of contamination

An alternative approach to the problem of soil contamination involves the estimation of the percentage of extraneous mineral matter and the subsequent application of correction factors to the results obtained from contaminated samples. A study of the relevant literature revealed that several methods of estimating soil contamination had been used in the past. With a single exception, all such methods are based upon the determination, in the suspected sample, of some constituent essentially associated with soil minerals. An increased value for this constituent above an accepted normal for plant material is then taken as indicative of soil contamination. If sufficient data concerning the usual levels of the constituent found in plants and soils are available, an estimation of the degree of contamination is then possible.

The most commonly determined constituent for use in the above technique is total silica. Woodman *et al.*[3] suggested this element as being suitable for the calculation of correction factors to be applied to the results of conventional feeding stuff analysis. These authors assumed a value of 1·72% of silica in uncontaminated herbage, while many subsequent workers have adopted a value of 2%.

A less widely known variant of the silica method is that of Askew,[22] which depends upon his contention that the elaborated silica of plants is almost completely soluble in 2% sodium hydroxide, whereas the silica in soils is considerably more resistant to this treatment. A determination of the ' insoluble silica ' content of the suspected sample, along with that of the soil itself, allows of an estimate of the degree of soil contamination.

Aluminium is another element whose presence in a herbage sample in any quantity may be accepted as evidence of contamination. Aston[23] suggested this procedure after comparing it with the total silica method. Aston considered a sample free from contamination if its alumina (Al_2O_3) content was below 0·05%, i.e., approximately 250 p.p.m. of Al. Shorland[5] has given considerable attention to the development of the method as a quantitative index of contamination.

Recently Mitchell[7] has suggested that extraneous mineral material in herbage samples is best detected by a determination of the titanium content of the material. Mitchell claims that as normal plant materials usually contain titanium of the order of 2 p.p.m. and soils about 10,000 p.p.m., the method is capable of detecting minute contaminations. Unfortunately the methods available for the determination of this element, other than the spectrographic method as used by Mitchell, leave much to be desired, and the technique is unlikely to find general application.

In the course of investigating some of the contamination errors in mineral analysis of herbage, Tomlinson[24] devised a direct method for the estimation of the amount of adhering soil on wet grass material. This consisted of washing the sample free from soil in a glass vessel, removing the plant material, oxidizing any soluble organic matter with peroxide and finally filtering off the residual soil which was weighed after ignition. Tomlinson claimed that this method, while liable to under-estimate slightly the percentage contamination, at least did represent a basic minimum value upon which reliance could be placed.

In the present study, all the procedures mentioned above, with the exception of the titanium and the direct washing methods, have been compared. It is convenient to present and discuss the results under sectional headings as follows.

(*a*) *Total silica method* (*Woodman*).—The total silica values of an artificially contaminated series of samples were determined by the conventional procedure. The results are presented in Table III.

An examination of the data presented in Table III shows that when soil contamination values are calculated using the assumed values of 2% and 80% of silica in plants and soils, respectively, the results obtained are invariably lower than the actual values. The error which arises from such a calculation is approximately 50% at the 2% contamination level, falling to about 8% as the contamination rises to 10%. While the latter error might be thought small, the former

Table III

Calculation of soil contamination based upon total silica content. (Results expressed on a dry-matter basis)

Actual soil contamination %	Silica content (SiO_2) %	Calculated soil contamination* %	Recovery* %	Calculated soil contamination** %	Recovery** %
0	1·16	0	—	0	—
2	2·84	1·05	52·50	2·01	100·50
4	4·65	3·31	82·75	4·18	104·50
6	6·01	5·01	83·50	5·80	96·67
8	7·88	7·35	91·88	8·04	100·50
10	9·34	9·18	91·80	9·79	97·60
Soil	83·60	—	—	—	—

* Values calculated on an assumed basis of 2% silica in uncontaminated plant material and 80% silica in soil.

** Values calculated using the determined silica contents of both plant and soil material, i.e., 1·16 and 83·60%, respectively.

cannot be considered so. In general it is the lower levels which may escape observation and which probably cause most erroneous results in trace-element analysis. Samples so seriously contaminated as to contain 10% of soil, could not pass unnoticed and would probably be discarded.

The accuracy of the total silica method is greatly enhanced by the use of the actual silica contents for the uncontaminated plant material and the soil. In this case the maximum error found in calculating the percentage contamination was about 3%. Excellent though the agreement may be using this procedure, its success depends entirely upon having available accurate knowledge of the silica content of the uncontaminated material, information impossible to obtain in the normal course of events. The true silica content of the soil is of course readily determined.

It will be apparent that the accuracy of the assumed-total-silica procedure is dependent upon the variation in actual and assumed silica contents of the uncontaminated plant material. Aston[23] has shown that the silica contents of pasture herbage are very variable, owing to inherent differences between plant species. He found, for example, that white and red clover had 0·15 and 0·11% of silica, respectively, while cocksfoot contained 1·82%. Thomas & Thompson[25] found silica contents ranging from 0·10 to 4·80% in different plant species, on samples which gave little indication of contamination by soil. Aston suggested that if the assumed-silica method was to be of any value, different standards would be required for different classes of plants. Satisfactory as this may prove in the analysis of individual species, it cannot find ready application when dealing with a mixed sward. In general, high total-silica values for samples of mixed herbage should be regarded as little more than an indication that contamination with soil has probably occurred.

(*b*) *' Insoluble ' silica method (Askew)*.—The results of the analyses of a prepared series of samples of known contamination, using Askew's method, are shown in Table IV.

Table IV

Calculation of soil contamination based upon ' insoluble ' silica content. (Results expressed on a dry-matter basis)

Actual soil contamination %	Insoluble silica (SiO_2) %	Calculated soil contamination %	Recovery %
0	0·48	0·64	—
2	1·92	2·55	127·50
4	3·69	4·63	115·75
6	5·07	6·73	112·17
8	6·66	8·57	107·13
10	7·91	10·48	104·80
Soil	75·42	—	—

The data shown in Table IV indicate that the plant silica, in this case, cannot be regarded as being completely soluble in 2% sodium hydroxide solution. This has resulted in the calculated soil contamination values being higher in every case than the true values. As in the previous method, the percentage soil recovery becomes more satisfactory at the higher levels of contamination. Askew obtained very low ' insoluble silica ' contents of herbage, but was not in a position to claim definitely that plant silica was completely soluble in the alkaline solution, due, as he himself explains, ' to the seeming impossibility of obtaining perfectly clean pasture samples '. Askew applied his method to many samples and used it to correct data from iron analyses.

In general, the ' insoluble silica ' method does not seem to offer any considerable increase in accuracy over the total silica method. Furthermore, in view of its uncertain basis, there appears little to justify its use without further study.

(c) *Methods based upon the determination of aluminium.*—The original procedure of Shorland[5] involved a double extraction of the plant material with hydrochloric acid, the separation of the aluminium in the extract as the hydroxide, and the determination of the metal by the development of a red lake with aurintricarboxylic acid in alkaline solution. However, in using this method difficulty was encountered in obtaining satisfactory duplication of results, even with pure aluminium solutions of known concentration. With such pure solutions excellent duplication and recoveries were obtained using the colour development procedure of E. M. Chenery (private communication) and this was adopted for all subsequent work.

The aluminium contents of a standard series of contaminated plant material were determined on both Shorland's hydrochloric acid extract and upon a normal nitric–perchloric acid extract such as would be obtained in wet-ashing herbage samples. However, in both cases a decreased extraction of aluminium was found as the percentage of soil increased. In addition, the nitric–perchloric mixture proved to be much less effective than the hydrochloric acid in removing the element from the soil. The latter fact is undoubtedly a serious objection to what might appear a most convenient procedure, involving no additional extraction to that normally prepared in trace-element analysis. The decreasing recoveries of aluminium from samples of increasing contamination, using either hydrochloric or nitric–perchloric acids, presents a further drawback to the use of these reagents. To calculate the percentage soil contamination would necessitate the construction of an extraction curve for the particular soil.

It was considered preferable, in view of the foregoing results, to aim for complete extraction of aluminium. To this end a series of contaminated samples was prepared in the manner previously described and the total aluminium contents determined after fusion with sodium carbonate. The procedure used was one suggested by Chenery, with slight modification. Details of the method are given in the Appendix.

The results shown in Table V clearly indicate the good correlation between actual and calculated percentage soil contamination using the sodium carbonate fusion method. The almost complete recoveries obtained show that complete extraction of aluminium has been effected at levels of contamination between 0 and 10%. The correlation coefficient between p.p.m. aluminium and contamination proved to be 0·999 while the regression coefficient was 271·5,

Table V

Calculation of soil contamination based upon total aluminium content. (Results expressed on a dry-matter basis)

Actual soil contamination %	Aluminium (Al) p.p.m.	Calculated soil contamination* %	Recovery %
0	105	0·37	—
2	560	1·98	99·0
4	1106	3·91	97·8
6	1742	6·16	102·7
8	2254	7·97	99·9
10	2762	9·77	97·7
Soil	28,280	—	—

* Values calculated using the determined aluminium content of the soil and assuming uncontaminated plant material to be free from this element.

indicating an increase of approx. 270 p.p.m. aluminium for every 1% soil contamination present. In view of the high correlation coefficient, and the excellent recoveries of aluminium obtained, the authors are unable to account for the apparent discrepancy between the aluminium content of the soil-free plant material and the other members of the series. The calculated aluminium content of this material, using the regression coefficient, is 63·9 p.p.m. In view of the data obtained, it would seem unwise to base calculated contaminations upon an assumed aluminium content of soil-free plant material, for this assumption might well introduce greater error than if plant aluminium is disregarded. At least one authority[26] is of the opinion that if all extraneous mineral matter is removed, the true aluminium of plant tissues is rarely more than 5 to 10 p.p.m.

The authors suggest that a herbage sample containing more than 200 p.p.m. of aluminium should be regarded with suspicion, particularly for trace-element analysis. Even at this level, analysis of such a sample may lead to serious errors of up to 50%.

While analysis of a seriously contaminated sample is to be strongly discouraged, the foregoing method is considered capable of providing an estimate of such contamination. In common with other methods employing the ratio of an element in plant and soil as its basis, certain assumptions are required which preclude any great accuracy. The most obvious is that which assumes the mineral matter on the herbage to be of the same composition as that of the soil in which it is growing. While this will not be the case with wind-blown and rain-splashed material, it is a valid assumption in the common and probably more serious contamination arising during sampling.

Assumed values for the aluminium contents of different soils may greatly limit the accuracy attainable by the above method. This is suggested by the aluminium contents of four soils shown in Table VI, along with the clay, silt and sand contents.

Table VI

The total aluminium contents of four soils of varying type. (Results expressed on a dry soil basis)

Soil No.	Aluminium (Al) p.p.m.	Clay %	Silt %	Sand %
1	28,280	12·4	18·3	68·8
2	53,600	28·3	21·0	47·6
3	57,320	34·0	18·2	44·2
4	40,800	24·2	15·5	59·4

Despite the limitations mentioned, the authors are in agreement with Shorland[5] that a method for estimating soil contamination based upon the aluminium content of the sample is preferable to one based upon other elements. Whether such a procedure is used only as a 'rejection test', or whether it is used for the calculation of 'correction factors', will depend primarily upon the level of contamination in the sample. There may be occasions when the application of such factors, with all their limitations, is preferable to leaving results which cannot be regarded as even approximations of the truth.

Discussion

In view of the very serious errors which may be introduced into mineral analyses of herbage, particularly for the trace elements, by the inclusion of small amounts of soil, it is obvious that great care must be taken in sampling. Contamination may, of course, arise prior to or during the collection of the sample. In the latter case, cutting excessively close to the ground, removing soil with the cutting implement, or allowing the cut material to come in contact with the soil, are all obvious causes and can be eliminated given reasonable care. Little, if anything, can be done when contamination occurs prior to sampling. This type of contamination may be caused by wind-blown soil, rain-splashed mud or by trampling of animals on fairly open swards. In industrial or semi-industrial areas there may be yet another cause of contamination, namely that from air pollution by dusts of various types. Data recorded on the King's College Farm at Nafferton, which lies just outside the industrial area, showed that in a single month 1 cwt. of water-insoluble matter fell per acre, of which about 20 lb. were inorganic in nature.

The whole subject of herbage contamination has considerable significance in the field of animal nutrition. It may perhaps be argued that, at least as regards herbage contaminated prior to cutting, the analysis obtained represents that of the material actually consumed by the animal. There is, however, insufficient evidence to conclude that such extraneous mineral matter is of equal availability for the animal as that contained in the plant. It is true that Rigg & Askew[27] found that drenching with soil extracts proved beneficial to sheep suffering from cobalt deficiency, but equally the experiments of Aston[23] and Shorland[5] suggest the complete unavailability of soil iron. It would, in the circumstances, appear advisable to disregard the possible nutritional value of such extraneous soil, and to consider only the amount of an element which is an integral part of the plant structure.

Appendix

Determination of total aluminium

Ammonium aurintricarboxylate reagent

Dissolve separately the following reagents, filter if necessary, then combine and make up to 1500 ml.

Ammonium aurintricarboxylate	0·75	g.
Ammonium acetate A.R.	200	g.
Concentrated hydrochloric acid A.R.	189	ml.
Gum acacia	15	g.

Method

Ignite 1 g. of the plant material in a small platinum crucible. When completely ashed, add about 0·5 g. of A.R. anhydrous sodium carbonate and raise the temperature slowly to bright orange heat. Maintain this temperature for 10 minutes, the crucible being half-covered by its lid. Cool and transfer to a 100-ml. beaker covered with a watch-glass. Rapidly introduce 1 ml. of concentrated nitric acid and about 3 ml. of distilled water. When the resulting vigorous reaction has subsided, remove the crucible and wash the contents into the beaker. Evaporate the resulting solution to dryness overnight to remove completely any excess nitric acid. Add about 10 ml. of water and 10 ml. of 1 : 50 nitric acid and heat on the water-bath for a further 20 minutes. If at this stage the silica is stained reddish-brown due to iron, bring the latter into solution by heating with 10 ml. of 0·5% thioglycollic acid until the silica is quite white. Filter the solution into a 100-ml. graduated flask, wash alternately with hot distilled water and two 10-ml. portions of 1 : 50 nitric acid, finally with cold water and then make to volume.

Transfer a suitable aliquot portion (containing approx. 10 to 15 μg. of Al.) to a 10-ml. graduated test-tube and add volumes of 1 : 50 nitric acid and 0·5% thioglycollic acid such that the final concentration of these reagents is 1 ml. of each. Add 2 ml. of ammonium aurintricarboxylate reagent, make to volume, and place the tube for exactly 15 minutes in a water-bath maintained at 90°. Transfer to a water-bath at 20°. Measure the colour of the red aluminium lake spectrophotometrically at 535 mμ. after a standardized time, between 6 and 48 hours after colour development.

Prepare a standard curve, using the same procedure as for the determination.

Note: Titanium, chromium and the rare earths are included to the extent of up to 1% (taken altogether) of the values obtained for aluminium.

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Received 25 July, 1955

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THE CRYSTALLIZATION OF COCOA BUTTER AND ALTERNATIVE FATS. I.—An Adiabatic Calorimeter and its Application to the Thermal Analysis of Cocoa Butter

By E. H. STEINER

An adiabatic calorimeter is described, suitable for investigating the thermal characteristics and phase composition of fats over a wide temperature range. Specific heats and differences in heat content are measured with maximum errors of 5% and 0·7% respectively. Sources of error in the calculation of percentages of solid and liquid phase from the data are discussed. Results are given for six samples of cocoa butter. Possible reasons are considered for the rise in the specific heat curve observed at about − 20°.

Introduction

Owing to present limitations in the supply of cocoa butter considerable interest centres round the production of alternative fats for use in chocolate and confectionery. A complete evaluation of the suitability of an individual fat for this purpose is not easy, the usual melting-point and cooling-curve data providing no guide to the physical behaviour of the stable fat. A fat cannot be entirely satisfactory as a substitute, however, if it does not show a plastic behaviour, over the working temperature range, similar to that of cocoa butter. Plasticity measurements themselves will be dependent on the treatment received during crystallization (e.g., degree of tempering) and a more fundamental requirement, therefore, is that the equilibrium proportions of liquid and crystallized phases at all temperatures should resemble those for cocoa butter.

The two main methods which are used in the study of phase equilibria in complex systems are the calorimetric and the dilatometric. Each has its own difficulties and a discussion of these in regard to measurements on fats is given by Bailey.[1] Since in the present work it has been found essential to commence measurements at low temperatures to ensure working with completely solidified fats, the calorimetric method has been adopted. As a preliminary to an examination of some of the alternative fats, the equilibrium proportions of solid and liquid phase

have been examined in a number of samples of the stable form of cocoa butter and, at the same time, various thermal data have been accumulated.

One of the difficulties in constructing a calorimeter suitable for measurements on fats lies in the relatively long time required for these materials to reach thermal equilibrium. Since it is necessary for the heating curve data to be obtained at a number of temperatures over a wide range it follows that a whole series of heat input and temperature measurements require to be made. Because of the low conductivity of the fats, experiments are liable to be very protracted. In situations of this nature it is necessary to adopt an adiabatic method, in which the thermal head between calorimeter and surroundings is kept to a minimum (ideally zero), otherwise corrections for heat loss may become as great as the quantity of heat measured.

Some of the most accurate measurements with a calorimeter which have been made on fats have been carried out by Bailey *et al.*[2] The heat input was measured electrically in a copper calorimeter containing a heating coil and a large number of discs for the purpose of conducting the heat rapidly into the fat. The calorimeter, filled with the melted fat, was mounted inside a heated jacket which was, in turn, placed in a vacuum-tight brass container. The whole could be immersed in any desired cooling bath. Determinations were made by plotting heat temperature curves for the calorimeter and jacket over the melting range and making subsequent corrections for heat interchange.

A much simpler procedure has been used by Vaeck[3] in a study of cocoa butter. Vaeck, who based his apparatus on a method of Straub & Malotaux,[4] used a small silver calorimeter in a water-bath which was maintained at a temperature always greater than that of the calorimeter by a constant amount. This heat gradient was itself used as the source of supply of heat to the calorimeter. As the temperature of the calorimeter, indicated by a thermometer, altered, so the temperature of the bath was adjusted by addition of hot water. This method has the advantage that cooling curves may also be plotted, but lacks the accuracy of Bailey's technique.

The present apparatus is based on that of Bailey, with certain modifications to promote easier operation. The calorimeter contains a heating coil in the centre carrying a series of vanes radiating into the fat for the rapid conduction of heat. Vertical vanes have been employed to permit ready filling with the fat either in the liquid or solid state.

The heating curve is obtained throughout the whole experiment in a series of runs during which the temperature rises by about 3° or less, depending on the extent of fusion occurring. To eliminate as far as possible the need for corrections for heat-loss, the jacket temperature is made to follow the calorimeter temperature almost exactly. Although heat leakages from the calorimeter have been kept low in designing the apparatus it has been necessary always to maintain the jacket temperature slightly above that of the calorimeter. Control of the jacket temperature is achieved by means of a copper–constantan thermocouple placed differentially across the jacket and calorimeter, and connected to a galvanometer. A second thermocouple is used to measure the temperature of the fat.

Experimental

Calorimetric apparatus

Calorimeter vessel.—The calorimeter proper consisted of a cylindrical copper vessel and lid approximately 3·3 cm. in diameter and 6 cm. high, with the outer surface of bright chromium plate to minimize radiation losses (Fig. 1). Fitting closely inside the vessel were 16 thin vertical vanes radiating from an inner cylinder of 1·4 cm. diameter. The inner cylinder, vanes and inner surface of the calorimeter were tin-plated. The vanes were perforated to reduce the mass and were regularly spaced so that at no point was the material in the vessel more than 3 mm. from a conducting surface.

Wound on a hollow core inside the inner cylinder was a nichrome heating coil of approximately 25 ohms, capable of carrying a current of 0·5 amp. The coil was connected to a 6-volt secondary cell in series with a 150-ohm variable rheostat. In addition to controlling the heating current this acted as an on–off switch.

The coil was sealed at the top with shellac and formed a single unit with the inner cylinder and vanes which could be easily removed from the calorimeter vessel for cleaning purposes.

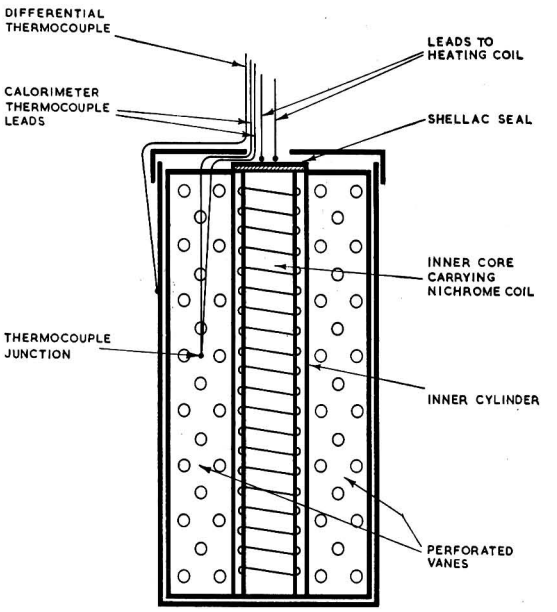

FIG. 1.—*Calorimeter vessel*

A small hole in the centre of the lid permitted the entrance of the leads to the heating coil and the thermocouple. The total mass of the vessel, vanes and heating coil was approximately 93 g.

Jacket.—This comprised a copper cylinder and lid of approximately 5 cm. diameter and 7 cm. height (Fig. 2). The inside surface was of bright chromium plate. Insulating cones of balsa wood were fixed inside to the bottom and sides of the jacket so that the calorimeter vessel was automatically spaced centrally when placed inside.

Separate heating coils of resistance totalling 50 ohms were wound round the sides, bottom and lid. The coils were mounted on mica and connected in series. Current to the jacket was supplied from a transformer delivering 24 volts a.c. and was controlled by a 150-ohm variable rheostat in series, acting also as a switch. Examination by thermocouples showed that the temperature was substantially uniform over the inside surface when a current was flowing through the coils.

The lid of the jacket was firmly held by means of three grub screws and carried a terminal block on its upper side. Both lid and block were provided with a central hole to enable the electrical leads from the calorimeter vessel and jacket to be brought out. Connexions were made at the terminal block from the external leads to the calorimeter coil and to the three jacket-heating coils. Access to the calorimeter vessel was readily obtained by disconnecting the calorimeter coil wires and two of the jacket heater wires from the block, when the lid of the jacket could be hinged back. In order to reduce heat leakage the leads to the calorimeter coil were wound several times round the outside of the jacket before being brought out through the outer container.

Outer container.—An outer container to hold the jacket was constructed from aluminium with approximate dimensions of 10 cm. diameter and 15 cm. height. This was lagged inside with felt and lined with brown paper. A stainless steel lid with a rubber gasket could be fastened down by means of four wing nuts to make a liquid-tight seal. The lid, which was provided with a 10-cm. long outlet tube for the electrical leads, was also lined on the lower side with felt and brown paper.

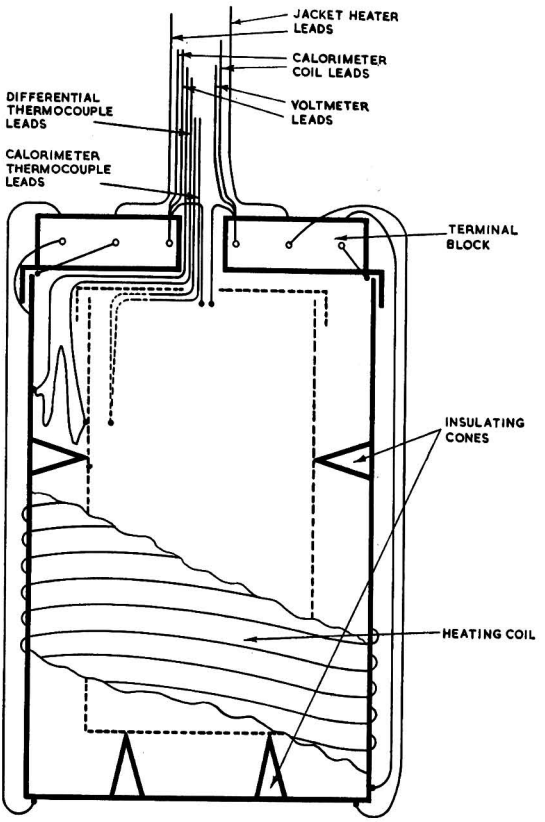

FIG. 2.—*Calorimeter jacket*

The calorimeter vessel, jacket and outer container will be collectively referred to as the calorimeter assembly. In carrying out measurements, the assembly was immersed in an appropriate liquid bath to a height of 1 cm. up the outlet tube of the outer jacket.

Temperature measurement

All temperature measurement was carried out with thermocouple junctions made from 40 s.w.g. copper and constantan. Calibration of the junctions was carried out over the range − 80° to + 100° by the National Physical Laboratory. Measurements of e.m.f. were made with a precision Two Dial Potentiometer with a lamp and scale galvanometer, capable of reading to 1 microvolt (approximately 0·025° C).

The junctions for the differential thermocouple were attached with adhesive tape to the outer calorimeter and inner jacket surfaces respectively. The leads were connected directly across a second lamp-and-scale galvanometer (referred to as the differential galvanometer). The sensitivity of this was such that 1 cm. deflection on the scale represented 0·1° difference between the calorimeter and jacket.

Measurement of heat input

The rate of heat input to the calorimeter was determined from the product of voltage and current in the heating coil. The current was found with the potentiometer by measuring the potential difference across a standard 0·1-ohm resistance (actual value 0·10002 ohm) in series with the coil.

The voltage across the coil was obtained directly by means of a moving coil galvanometer having a scale factor of 5·38 volts per 100 divisions.

Thus heat input to the calorimeter is given by the formula

$$H = V \times \frac{P}{0{\cdot}10002} \times \frac{t}{4{\cdot}186} = 2{\cdot}39VPt \qquad (1)$$

where H = heat input in mean calories
V = voltmeter reading in volts
P = potentiometer reading in volts
t = time in seconds
and 4·186 = mechanical equivalent of heat in joules/mean calorie.

Wiring of 40 s.w.g. was used in measuring the voltage in order to minimize heat losses by conduction from the calorimeter. These leads were encased together with the thermocouple leads in thin protective rubber tubing.

Calibration

Differential thermocouple.—Owing to the existence of unavoidable small losses of heat it was necessary to keep the jacket temperature slightly above that of the calorimeter in order to maintain the latter constant. Further, the magnitude of the required temperature-gradient increased with increasing difference in temperature between the calorimeter and the bath. The differential galvanometer was calibrated, therefore, to give the required scale-reading for a given calorimeter temperature (and bath temperature) and for various conditions of use, namely, with the calorimeter assembly immersed in acetone–solid CO_2 at $-78°$ approx., ice–salt mixture at $-20°$ approx., melting ice and water at 20° approx.

For calibration, the calorimeter was assembled empty with the thermocouple attached directly to one of the vanes, and immersed in the appropriate bath. When the temperature of the calorimeter vessel reached the bath temperature, a current was supplied for a short time to raise its temperature to some arbitrary value. Current to the jacket was then controlled to give a small fixed temperature-difference as indicated by the differential galvanometer scale-reading. This reading was held constant for 20 min. and any change in the calorimeter vessel temperature during this time was recorded. The procedure was then repeated with another differential galvanometer setting designed to give a temperature change in the opposite direction. By interpolation the correct setting to maintain the calorimeter vessel at constant temperature could be determined.

By carrying out calibrations with the calorimeter vessel brought to various temperatures it was possible to relate the differential galvanometer setting to temperature, and in all cases the relationships were found to be linear.

As the calibration was liable to change from one experiment to another, due to uncontrollable variation in the arrangement of the leads during the assembling of the calorimeter, a second-order error emerged from the use of an incorrect calibration. As this error was not negligible it was necessary to estimate its magnitude by determining the correction as a rate of gain or loss of heat to the calorimeter vessel per unit error in the differential galvanometer, and was found to be approximately 0·1 calorie/minute per 1-cm. scale error.

A correction of the calculated heat input during any interval was therefore determined from the above factor, and knowledge of the actual differential galvanometer setting and the proper setting for zero heat losses (or gains) used. The latter setting was always found by a calibration of the differential thermocouple at the end of each experiment (see under Procedure). All calibration factors obtained were within the limits of 0·1 to 0·2-cm. scale setting per 1° temperature-difference between the bath and calorimeter vessel.

Thermal capacity of the calorimeter vessel.—The thermal capacity of the calorimeter vessel (including heating coil, inner cylinder and vanes) was determined by carrying out a series of heat input–temperature measurements with the vessel empty. The thermocouple junction was attached to one of the vanes and the assembly immersed in an appropriate bath. Details of the procedure used and the calculations of corrected heat inputs were identical with those

described below under ' Procedure ' except that after each 10-min. heating period a further 2 min. only was allowed for equilibrium (this was, in fact, established almost instantaneously).

The ratio of heat increment to temperature rise was calculated for each input of heat over the range -70 to 60°. From measurements obtained in two duplicate runs the thermal capacity was found to vary with temperature in a linear fashion given by $w = 10{\cdot}1 + 0{\cdot}0{\cdot}014\ \theta$, where w = thermal capacity at temperature θ° C.

Procedure

The calorimeter vessel was filled with the fat (about 25 g.) under test, generally grated when solid, or, if sufficiently plastic, merely pressed into the spaces between the vanes. The thermocouple junction was then inserted and held in place by carefully pressing the fat round the wires. The vessel was mounted inside the jacket, connexions made at the terminal block to the heating coils, and the whole put in the outer container. Where measurements were required at low temperature the assembly was left overnight at that temperature in order to reach equilibrium.

Connexions to the potentiometer and galvanometer were made and the temperature of the calorimeter vessel determined just prior to the experiment. Current was then supplied to the calorimeter heating coil at a rate sufficient to produce a rise of 3° or 4° C in 10 min. in the solid fat. The necessary heat input for this was about 8 calories per min. or approximately 0·5 watt (corresponding to a potential across the coil of 3·5 volts). This heating rate was increased during the melting interval as the rate of temperature rise became considerably reduced. Simultaneously with the heating of the calorimeter, current was supplied to the jacket coil and controlled manually by the variable rheostat.

Heating of the calorimeter vessel was maintained at a constant rate for 10 min., during which time two readings of voltage and current in the coil were taken. Where necessary, an average value was used in calculating the heat input. After cutting off the current to the calorimeter coil, the contents were allowed to reach thermal equilibrium and the temperature again measured. Five min. were found to be sufficient for this, after which time the current to the calorimeter coil was again switched on. Current to the jacket coil was maintained continually throughout the whole period, during which time the differential galvanometer setting was held constant by control of the variable rheostat. The differential scale-reading adopted was that expected to be approximately correct for the mean calorimeter temperature during the 15-min. period.

In this way a series of heat input–temperature measurements was made over a whole temperature range. Where measurements commenced at -78° in an acetone–CO_2 bath the temperature of the calorimeter vessel was taken up to 0° during one day and from 0° to a temperature beyond that at which the fat completely melted (about 50°) the following day, using an ice-bath.

At the end of each day's measurements the calibration of the differential thermocouple setting was checked. A single point (i.e., at the final temperature reached in the series of measurements) was sufficient to establish the appropriate line, since the calibration was known to be linear.

Calculation of results

(1) The input of electrical energy into the calorimeter vessel between one temperature measurement and the next was calculated by equation (1).

(2) The heat input was corrected for errors in the differential galvanometer setting as follows: The correct differential galvanometer setting for the average temperature during each heat input was determined from the calibration established at the end of the experiment. The actual setting used was deducted and the difference multiplied by 1·5 (see above) since a total time of 15 min. elapsed. The quantity was then added algebraically to the uncorrected heat input to obtain the correct value ΔH.

(3) Thermocouple readings in microvolts were converted to °C and the increase in temperature $\Delta\theta$, corresponding to each corrected heat input, was computed.

(4) The increase in heat content per gramme of fat was calculated for each temperature interval as

$$\Delta h = \frac{\Delta H - w\Delta\theta}{m} \qquad (2)$$

where m = mass of fat and w = thermal capacity of calorimeter at mean temperature θ. For convenience in comparing different fats, a heat content of zero was arbitrarily assumed for the melted fat at 50° (taking heat contents below 50° as negative). As it was impossible to take a reading at exactly 50° the heat contents at the two experimental temperatures on either side of 50° were obtained by proportionality. The heat contents at the other temperatures (termed relative heat content) were calculated by proceeding backwards and forwards from these two initial values, respectively subtracting and adding the increments Δh.

Where the measurements were made in separate stages from − 70° to 0° (approx.) and 0° to 50° (approx.) it became necessary to join the two series together in the neighbourhood of 0°. Experimentally, the final temperature reached in the − 70° to 0° run was made to overlap the commencing temperature of the 0° to 50° run. The final heat increment in the first run which would have been required in order to end at the starting temperature of the second run was then calculated in proportion.

(5) Specific heats in the solid and liquid states were calculated from adjacent temperatures as

$$s = \frac{\Delta h}{\Delta \theta} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Accuracy of measurements

Error in heat input.—The heat contents, ΔH, are calculated from four independent experimental factors, viz.: voltmeter reading, potentiometer reading, time, and differential galvanometer scale-reading. Typical values of the first three of these quantities in the standard heating method are 70 (scale-reading), 0·014 (volt), and 600 (seconds). Maximum errors made in taking these readings are ± 0·2 (scale-reading), ± 10 (microvolt), and ± 1 (second), leading to an overall maximum error of ± 0·4 calories in the uncorrected heat input.

If an error of ± 0·4 scale deflection is assumed in computing the differential thermocouple correction, the error introduced into the correction term over 15 min. is ± 0·6 calories. The maximum combined error in the corrected heat input is, therefore, ± 1·0 calories.

Error in temperature.—An error of ± 1 microvolt, corresponding to ± 0·025° C, may be taken as the maximum error in determining temperature. The maximum error in $\Delta\theta$ for a given heat input is, therefore, ± 0·05° C.

Specific heat.—From the above, the maximum errors in specific heat due to heat input and temperature are $1{\cdot}0\frac{\partial s}{\partial H}$ and $0{\cdot}05\frac{\partial s}{\partial \theta}$. Since, from equations (2) and (3),

$$s = \frac{1}{m}\left[\frac{\Delta H}{\Delta \theta} - w\right]$$

the maximum total error may be calculated as

$$\pm \frac{1}{m\Delta\theta}\left[1 + 0{\cdot}05\frac{\Delta H}{\Delta \theta}\right] \quad \text{or} \quad \pm \frac{ms + w}{m\Delta H}[1 + 0{\cdot}05(ms + w)]$$

Inserting typical values for m, s, w and ΔH it is found that the maximum percentage error in specific heat remains roughly constant at about 5% over the range 0·25 to 2·0.

Relative heat content.—The error in Δh due to temperature measurement is negligible, but each heat measurement contributes a maximum error of $\pm \frac{1{\cdot}0}{m} = 0{\cdot}04$ calorie/g. A limit to the excess of positive over negative errors (or vice versa) can be deduced from statistical considerations, employing the binomial distribution. Since no result is likely outside a range of 4 times the standard error, a maximum error in h, from a total of n heat input measurements, can be assessed as $2\sqrt{n} \times 0{\cdot}04$. For all measurements below about 30°, the maximum percentage error in relative heat content equals 0·7% approximately.

Latent heat of fusion of ice.—The accuracy of the apparatus was checked experimentally by making a series of measurements on ice and water from − 10° to + 15°, approximately, using an ice–salt bath.

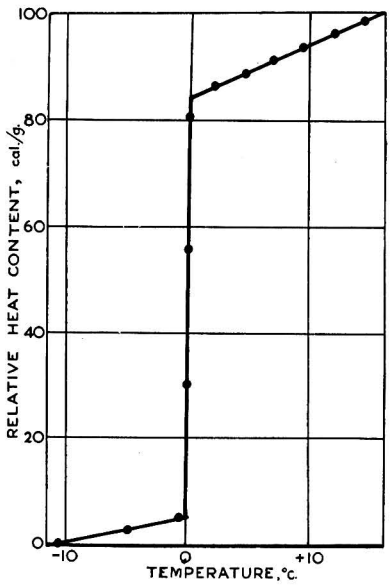

FIG. 3.—*Latent heat of fusion of ice*

On plotting the relative heat contents, calculated from an assumed zero value at the commencing temperature, against temperature, Fig. 3 was obtained, from which the latent heat of fusion of ice is derived as 79·5 mean calories per g. in agreement with the accepted figure (79·7 mean calories/g.). The specific heat measurements are accurate to within ± 2%, although this is insufficient to exhibit the effect of temperature on the specific heat of water, which appears constant within the error of the method.

Cocoa butters used

Six samples of pressed cocoa butter (A to F), as used by manufacturers of chocolate in this country, have been examined in the calorimeter. Samples E and F were prepared from alkalized nib, while sample C was of interest in being over 22 years old. In general all the samples were prepared at least several months prior to examination and no further stabilizing treatment was deemed necessary, the calorimeter being filled directly with the grated fat.

Example.—Table I illustrates the calculation of the heat content of a sample of cocoa butter (Sample A) over a portion of the experimental run on either side of 0°. Current and voltage readings are omitted, so that the uncorrected heat inputs are shown directly in column 3. The temperatures given were measured after 15-min. intervals, representing 10 min. heating plus 5 min. to reach equilibrium. Data for the determination of the correct differential galvanometer setting at the end of the run in the acetone–CO_2 bath are shown in Table II.

Table I

Calculation of relative heat contents. Part of data from a sample of cocoa butter heated from − 76° to + 60°. (Weight of sample = 23·93 g.)

Bath	Calorimeter temp. (° c)	Heat input uncorr. (cal.)	Differential galvanometer setting (cm.) Actual	Corrected	Correction to heat input (cal.)	Heat input ΔH (cal.)	$\frac{\Delta H - w\Delta\theta}{m}$	Relative heat content h
Acetone–solid CO_2	− 8·44	—	—	—	—	—	—	− 63·0
	− 4·77	75·1	8·9	7·2	+ 2·6	77·7	1·64	− 61·4
	− 1·44	75·2	9·5	7·5	+ 3·0	78·2	1·80	− 59·6
	+ 2·09	78·7	10·0	7·8	+ 3·3	82·0	—	—
Ice	+ 0·15	—	—	—	—	—	0·85	− 58·7
	3·13	69·3	0·5	0·4	+ 0·1	69·4	1·59	− 57·1
	5·72	68·8	0·9	0·7	+ 0·3	69·1	1·75	− 55·4

In Fig. 4, curve A, the continuous curve shows the relation between relative heat content and temperature for cocoa butter A over the whole range of the experiment. That portion of the curve from − 70° to − 20° represents heat absorbed in raising the temperature (without fusion) of the completely solid fat, the slope measuring the specific heat. Similarly the curve from about + 35° upwards represents the heat absorbed due solely to the specific heat of the completely liquid fat. Intermediately, the course of the curve is the result of the combined effects of heat absorbed due to specific heat and to heat of fusion and provides the means of estimating, approximately, the proportion of solid and liquid phases at any temperature.

Table II

Determination of correct differential thermocouple setting for data of Table I (acetone–solid CO_2 bath)

Bath	Time (min.)	Calorimeter thermocouple (microvolts)	Rate of increase (μV/min.)	Differential galvanometer setting (cm.)	Equilibrium setting (cm.)
Acetone–CO_2	0	97			
	5	98			
	10	99	+ 0·20	9·0	
	15	100			
	20	101			
					7·8
	0	100			
	5	100			
	10	99	− 0·05	7·5	
	15	99			
	20	98			

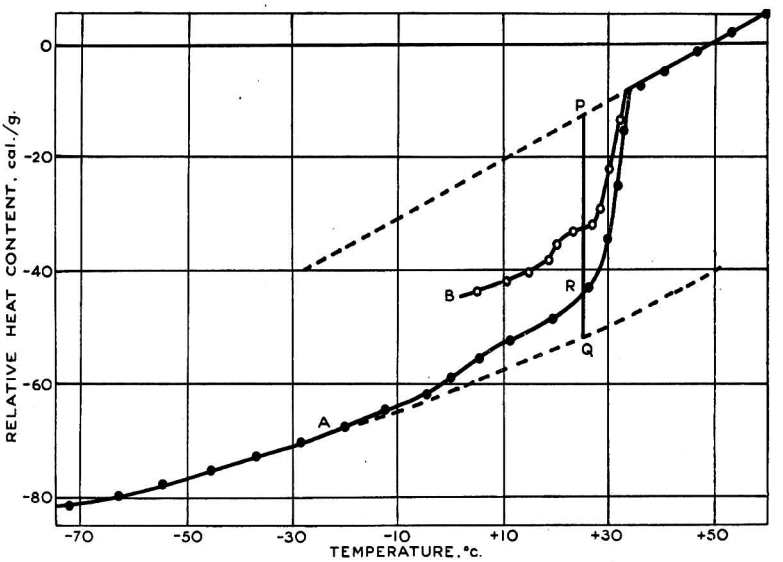

FIG. 4.—*Heat content and temperature of cocoa butter (Sample A)*

Curve A Stable form
Curve B α-form

Percentage of solid and liquid phases

In estimating the phase composition the usual procedure is first to extrapolate those parts of the heat content–temperature curve due to completely solid and completely liquid fat. This can be done with accuracy since the specific heat–temperature relationships over these ranges are in general found to be linear. For cocoa butter A these relationships (Fig. 5) are

$$\left.\begin{aligned} s &= 0{\cdot}365 + 0{\cdot}0016\theta \text{ for solid fat} \\ \text{and} \quad s &= 0{\cdot}51 \text{ for liquid fat} \end{aligned}\right\} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

respectively, where s = specific heat and θ = temperature (° c). The corresponding relationships between heat content and temperature are found by integration, the constants being calculated by reference to the experimental data. For the above sample $h = -80{\cdot}4$ when $\theta = -60{\cdot}0$, and by definition, $h = 0$ when $\theta = 50$, leading to the relations $h = -61{\cdot}4 + 0{\cdot}365\theta + 0{\cdot}0008\theta^2$, and $h = -25{\cdot}5 + 0{\cdot}51\theta$, for the solid and liquid fat, respectively. The extrapolated curves, shown by the broken lines in Fig. 4, are drawn in accordance with these equations.

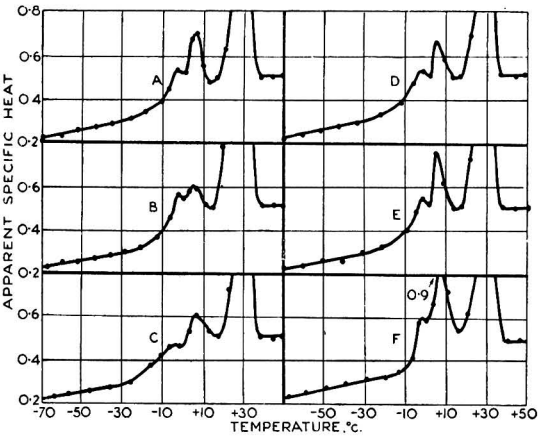

FIG. 5.—*Specific heat and temperature of cocoa butter*

	Solid fat	Liquid fat
Sample A	$s = 0{\cdot}365 + 0{\cdot}0016\theta$	$s = 0{\cdot}51$
Sample B	$s = 0{\cdot}37 + 0{\cdot}0013\theta$	$s = 0{\cdot}51$
Sample C	$s = 0{\cdot}365 + 0{\cdot}0013\theta$	$s = 0{\cdot}51$
Sample D	$s = 0{\cdot}38 + 0{\cdot}0016\theta$	$s = 0{\cdot}52$
Sample E	$s = 0{\cdot}37 + 0{\cdot}0012\theta$	$s = 0{\cdot}51$
Sample F	$s = 0{\cdot}385 + 0{\cdot}0014\theta$	$s = 0{\cdot}50$

With the extrapolated lines as shown, the proportion of solid or liquid phase is obtained by drawing a vertical intercept at the required temperature, e.g., the line PQ at 25°, cutting the curve at R in Fig. 4. The proportion of liquid is then RQ/PQ. Values obtained in this way for the above sample of cocoa butter are shown in the last column of Table III (to the nearest 0·5%).

Accuracy of the calculation

Although the above procedure is the one generally adopted, for the interpretation of both calorimetric and dilatometric data, it is strictly valid only if the extrapolated lines for both the completely liquid and completely solid fat are parallel and, also, if the latent heat of fusion (or melting dilatation) is constant over the melting interval. In practice neither of these conditions is fulfilled exactly and it is desirable to examine to what extent errors are introduced. Since an increasing amount of liquid phase forms as heat is applied over the melting range, the quantity of heat which causes temperature rise alone will actually be represented by a line intermediate in slope between the extrapolated solid and liquid lines. By making use of the approximate values of the proportion of liquid phase found graphically by the intercept method, it is possible to compute the specific heat at any temperature in the melting range and arrive at a more accurate value for the heat absorbed in causing fusion.

Let θ_1 and θ_2 be two adjacent measured temperatures (Table I) and let $\bar{x}$ be the mean proportion of liquid phase in the interval. Then the quantity of heat per g. of fat causing change in temperature alone will be

$$\left[0{\cdot}365 + 0{\cdot}145\bar{x} + 0{\cdot}0016(1 - \bar{x})\frac{\theta_1 + \theta_2}{2}\right](\theta_2 - \theta_1)$$

As an approximation to $\bar{x}$ the value of the proportion of liquid phase at temperature $\frac{\theta_1 + \theta_2}{2}$, obtained by the intercept method, may be substituted. Subtracting the numerical value of the above expression from the known total heat absorbed per g. of fat the quantity of heat causing fusion in the interval is found. This quantity may be conveniently designated $L\Delta x$ where L is the latent heat of fusion and Δx the increase in the proportion of liquid phase.

Table III, column 4, gives the values of $L\Delta x$ calculated in this manner for the various temperature intervals from 0·15° to 37·20° from the data for cocoa butter A.

Table III

Calculation of the proportion of liquid phase in cocoa butter A assuming constant and variable latent heat

Temp. (° c)	Approx. mean proportion of liquid in temp. interval	Heat absorbed by cocoa butter (cal./g.)		Percentage of liquid phase at $\theta°$			
		Causing temp. rise	Causing fusion	Latent heat constant (38·27 cal./g.)	Latent heat variable (29 to 54 cal./g.)		Intercept method
θ	$\bar{x}$		$L\Delta x$	x%	$\bar{L}$	x%	x%
0·15				4·4	29·4	5·4	5·5
3·13	0·07	1·11	0·48	5·7	29·5	6·9	7
5·72	0·09	0·99	0·76	7·7	29·7	9·4	9
8·24	0·11	0·98	0·81	9·8	29·9	12·0	11
11·41	0·12	1·25	0·50	11·1	30·0	13·7	12
15·42	0·13	1·62	0·29	11·9	30·1	14·6	13·5
19·32	0·14	1·60	0·36	12·8	30·2	15·8	14·5
23·39	0·15	1·70	0·88	15·1	30·3	18·6	17
26·49	0·18	1·33	1·70	19·5	30·7	24·0	21
28·62	0·24	0·93	2·52	26·1	31·5	31·8	27·5
30·02	0·32	0·63	3·94	36·4	32·8	43·5	36·5
30·92	0·42	0·41	4·35	47·8	34·8	55·6	46·5
31·62	0·53	0·34	4·52	59·6	37·5	67·3	57·5
32·31	0·64	0·33	4·54	71·5	40·6	78·0	70
33·04	0·76	0·36	4·49	83·3	44·5	87·8	83
33·98	0·90	0·47	4·29	94·5	49·7	96·3	95
37·20	1·00	1·64	2·12	100·0	54·0	100·0	100

It is not possible to proceed with the calculation of the percentage of solid or liquid phase unless some assumption is made concerning the latent heat of fusion. If the products $L\Delta x$, obtained as above, are summed over the complete melting range (from $-24{\cdot}5°$ upward) their values are found to total 38·27 calories/g. If the latent heat of fusion is assumed constant, equal to this numerical figure, values of Δx, and hence of x by summation, may be computed. These are the values shown as a percentage in column 5 of Table III. It is clear that the graphical procedure is reasonably accurate (assuming constant latent heat) and that no appreciable error arises from failure to allow for variation in the specific heat as melting proceeds. This is due to the fact that the extrapolated lines of the solid and liquid cocoa butter are almost parallel.

Variation in latent heat

A more serious source of error lies in the assumption of constant latent heat during melting. The latent heats of different glycerides increase with length of the fatty acid chain and, to an even greater extent, with degree of saturation, although actual data are very incomplete. Bailey[6] gives values of 29, 53·1 and 54·6 for the latent heats of triolein, tripalmitin and tristearin (stable forms), respectively. Since in general the composition of the solid phase during melting changes continually towards a greater proportion of the more saturated and longer chain (higher melting) glycerides, it follows that the latent heat of fusion per g. of solid fat should increase progressively. Thus a functional relationship of the type $L = a + bx + cx^2$ may be assumed between the latent heat per g. of solid fat at any temperature and the proportion of liquid phase at that temperature.

Extreme values for the latent heat at each end of the melting range of cocoa butter and many other fats (except for those rich in linoleic or linolenic acids) may be taken as 29 and 54 cal./g. In the absence of knowledge as to changes in the composition of the solid phase and its latent heat during melting, it is of interest to examine the effect on the calculation of liquid phase of a variation of the above order. Applying these extreme values of the latent heat to the cocoa butter data the constants a, b and c in the above equation can be evaluated, since $L = 29$, when $x = 0$, $L = 54$ when $x = 1$ and $\int_0^1 L\,dx = 38{\cdot}27$. Solving the resulting simultaneous equations gives $a = 29$, $b = 5{\cdot}8$ and $c = 19{\cdot}2$.

In Table III, columns 6 and 7, the relationship $L = 29 + 5{\cdot}8\bar{x} + 19{\cdot}2\bar{x}^2$ has been used to calculate the latent heat of fusion over each temperature interval (average proportion of liquid phase $= \bar{x}$), hence, also, Δx and x% as previously. It is clear that the existence of a variable latent heat of fusion can appreciably influence the calculation of the solid and liquid phases. The assumption of constant latent heat leads to low percentages of liquid phase, although it is of

interest that in the lower part of the range the graphical method gives results in better agreement with those assuming variable latent heat than does the calculation which allows for variation in specific heat.

In the case of most natural fats it is unlikely that the variation of latent heat will be nearly as great as supposed above, since this involves the melting (and, therefore, existence) of appreciable quantities both of tri-unsaturated and tri-saturated glycerides. Such a situation might arise with mixed fats, however, and the intercept method of calculating percentages of liquid phases must be regarded as giving low results with a maximum error, over the middle part of the melting range, of about 20% (of the liquid phase). As far as the results for cocoa butter are concerned, the variation in latent heat is probably less than half that assumed here, in which case a calculation along the above lines indicates that the percentages of liquid obtained graphically will not be in error by more than 5 to 10% (of the liquid phase).

Results

Relative heat content

When plotted against temperature, the relative heat contents of all the cocoa butters followed Fig. 4 (curve A) closely. Over the range − 70 to + 25° the maximum variation found was about 2 cal./g., reflecting a small, though real, difference in behaviour between the samples. Some typical values of heat content are shown in Table IV.

Table IV

Thermal characteristics of cocoa butter

Sample	Latent heat at mid-fusion (cal./g.)	Temp. of mid-fusion (° c)	Temp. of 100% liquid phase (° c)	Relative heat content* (cal./g.) at		
				− 60°	0°	20°
A	38·8	31·1	34·4	− 80·4	− 58·8	− 47·4
B	39·1	31·4	34·2	− 80·8	− 59·1	− 47·3
C	37·7	31·0	33·6	− 78·9	− 57·7	− 46·5
D	38·5	30·8	34·0	− 80·2	− 58·8	− 47·5
E	37·9	30·6	34·0	− 79·5	− 58·3	− 47·0
F	37·3	30·2	33·7	− 80·5	− 58·4	− 45·0

* Heat content at 50° assumed zero

Specific heat

The slope of the heat content–temperature curve at any point measures apparent specific heat. Variations in the latter over the range − 70° to + 50° are exhibited in Fig. 5. Below − 20° and above + 35° the points lie on straight lines, for which equations are given, representing true specific heats of the completely solid and completely liquid fat. The existence of two maxima, at − 2° and + 6° approximately, before the main rise due to the major part of melting, appears to be quite characteristic of cocoa butter.

Phase composition

The percentages of liquid phase have been calculated for each sample by the intercept method at specific temperatures over the range 0° to 15° and the results plotted in Fig. 6. It is of interest that cocoa butter, which is noted for its properties of brittleness and good ' snap ', gives rise to a curve which is relatively flat at normal temperatures, showing not more than 10 to 15% liquid phase, but which rises steeply above 25°. All the samples examined exhibited this same general characteristic.

Discussion

A close examination of the heat content curve for cocoa butter (Fig. 4) reveals that it is composed of separate segments. Thus between − 20° and melting, the curve rises in three steps: from − 20° to 0°, from 0° to 20° and from 20° to the temperature of final melting (corresponding to the maxima in the specific heat curve). The existence of segments in both calorimetric and dilatometric curves has been recognized before as characteristic of plastic fats[7] and presumably corresponds with the appearance of phase boundaries in the phase diagram.

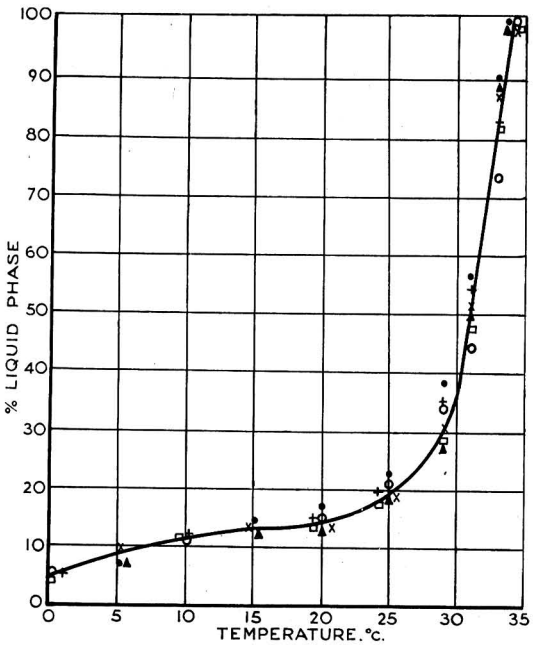

FIG. 6.—*Percentage liquid phase and temperature of cocoa butter*

What is remarkable, however, is the apparent melting of cocoa butter which commences at a temperature of — 20°. The lowest melting of the various constituent glycerides of cocoa butter as given by Hilditch & Stainsby[8] and Meara[9] are stearodiolein and palmitodiolein. These components, which together comprise about 20% of the cocoa butter, both have melting points of the order of 20° in the stable state. In order to account for melting at the low temperature the presence of some component such as trilinolein (melting point — 13°)[10] would be required. The extent of the melting over the range — 20° to 0°, according to Fig. 3, is only 2 or 3% and it is interesting to note that, although Hilditch & Stainsby do not consider the existence of triunsaturated glycerides probable in cocoa butter, they do admit the presence of 2% of linoleic acid.[8]

There are, nevertheless, other explanations which may account for the apparent melting observed. In cooling down to — 70° it is possible that part of the fraction still liquid at room temperature crystallizes in an unstable form. Thus, for example, in its least stable state palmitodiolein shows a melting point of — 13°.[11] The existence of unstable polymorphic forms with low melting points is well established for pure glycerides[12] and there is abundant evidence for their presence in cocoa butter.[13–15]

A different fact which may have a bearing is that premelting has been shown to occur in the heating of certain long chain compounds even when these are in a state of high purity.[16, 17] The specific heat–temperature curve of these compounds was found to show a rise prior to the true melting point by as much as 15°. This behaviour is attributed to the development of flaws in the crystal lattice and possibly also to the onset of rotation in the molecular chains.

The second rise, to a maximum at 6°, is more strongly indicative of melting, and liquid phase may certainly be considered to exist above about 0°. The fact that a deviation from the curve for solid cocoa butter commences at — 20°, however, emphasizes the importance of starting from a low temperature when the proportion of solid and liquid phases are required.

Conclusions

Whilst the variation with temperature of the proportion of solid or liquid phase is important in relation to the plastic properties of a fat the calculation of these proportions is subject to some uncertainty, as discussed above. The heat content curve itself is valuable in studying polymorphic forms and transformations. Thus curve B, Fig. 4, represents the relative heat content of cocoa butter after rapid cooling from 70° to 0° (α polymorphic form). If measurements are made on the fat in the stable form, the heat content curve, or the derived specific heat curve, is a characteristic of the fat over a wide temperature range which may provide a useful analytical criterion if other methods fail.

The heat content measurements also enable an accurate determination to be made of the temperature corresponding to 100% liquid phase. Here it is only necessary to obtain measurements over a small temperature range on either side of melting, the temperature of 100% liquid phase being given as the point of intersection of two lines.

Another useful constant is the apparent latent heat at mid-fusion, obtained as the length of the vertical intercept between the extrapolated solid and liquid lines at a temperature where it is bisected by the heat content curve. Although arbitrary, since the latent heat of a mixture of glycerides cannot be a constant figure, the latent heat at mid-fusion represents an average value for the heat of fusion per gramme of completely solidified fat. Table IV shows the latent heats and temperatures at mid-fusion for each of the samples of cocoa butter. Also included are the temperatures of 100% liquid phase and relative heat contents at three different temperatures.

The latent heat values given are higher than that recently found for stable cocoa butter by Vaeck[3] (36 cal./g.), presumably due to the fact that his measurements commenced at 0° instead of − 70°. If that part of the relative heat content from 10° to 20° is extrapolated, a value of 32 cal./g. is obtained for the latent heat. Since the heat content curve over this range is roughly parallel to the extrapolated liquid line (Fig. 4), it follows for practical purposes that, in calculating the heat required to melt cocoa butter from room temperature, a specific heat of 0·5 and a latent heat of 32 cal./g. may be assumed.

Acknowledgments

The author wishes to thank the Director and the Council of the British Food Manufacturing Industries Research Association for permission to publish this paper. Grateful acknowledgment is due to a former colleague, Dr. W. J. Stainsby, and to Dr. E. Griffiths of the National Physical Laboratory, for help in the design of the calorimeter, also to Mr. A. G. Edwards for carrying out most of the experimental work. The construction of the calorimeter assembly was carried out by Messrs. Nash & Thomson, Tolworth, Surrey.

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Received 11 March, 1955

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FUMIGATION OF AGRICULTURAL PRODUCTS. XI.*— Sorption of Mercury Vapour by Wheat†

By B. S. GORRINGE

A more sensitive modification of an ultra-violet absorption method for detecting mercury vapour is described, capable of detecting 10^{-10} g. of mercury vapour in approximately 50 ml. of air. No sorption of mercury vapour could be detected on Pyrex glass vessels. Sorption on wheat is influenced much more by changes in the moisture content than by changes of temperature within the normal range of both factors. Most of the mercury sorbed is chemically combined, but with drier grain some can be recovered by airing. The reaction of mercury vapour with wheat continues even after two years' exposure to the fumigant. Concentrations of mercury lethal to normal grain weevil eggs are likely to extend for two feet around foci of diffusion in a mass of grain. Health hazards appear to be slight.

In an earlier paper[1] the development of an apparatus for detecting mercury vapour in air was outlined, as was the use of the apparatus for experiments on the sorption of mercury vapour on wheat.

One serious disadvantage of this detector was its inability to work satisfactorily at and below the range 0·1 mg. of mercury per cubic metre of air. This concentration has been reported as the maximum permissible contamination in which it is considered safe to work for long periods.[2] Of the possible methods of increasing the sensitivity of the apparatus, that finally adopted was a newly designed amplifying circuit of greatly increased sensitivity.

Experimental

Apparatus

Design of amplifying circuit.—The circuit was designed to be of high sensitivity and arranged to balance at full light intensity, i.e., with a clean light absorption tube. Any small decrease in intensity at the photocell, due to traces of mercury, caused the circuit to go out of balance very rapidly. As can be seen from Fig. 1 it was a balanced bridge, and two matched valves were used, operating on the linear portion of their characteristics. Particular attention was paid to anode–cathode insulation, and for this reason the QVA 38 photocell was supplied unbased. The high resistor, R_5, was mounted on porcelain insulators and the metal shielding on the valves removed to reduce grid–earth leakage. In operation, any change in the illumination of the photocell, P, resulted in a change in the grid current of V_1 which was recorded on M_I. The current gain between the grid current of V_1 and M_I was of the order of 10^4.

To operate the amplifier after an initial warming up period the H.T. (high tension) voltage was adjusted to 210 volts as registered by the meter M_p using the variable resistor R_{12}. The cathode currents in the two valves as shown on meters M_A and M_B were then equalized using R_{11}. Any change in the illumination of the photocell P then resulted in a change in the grid current of V_I which appeared as a voltage which was recorded on the indicator meter M_1. This indicator meter was fitted with a six-position range switch giving three linear ranges, position 3 giving highest sensitivity and positions 2 and 1 lower sensitivity by switching in resistors R_8 and R_7 respectively. Positions 4 and 5 gave two non-linear ranges by incorporating the meter rectifier and resistor R_6.

Construction of the apparatus.—A block diagram of the apparatus is shown in Fig. 2. Since its general features have already been described,[1] only the additions made to the original detector will be briefly mentioned.

The light-absorption tube (LA) was reduced in diameter from 1½ in. to 1 in., thus diminishing its volume from 113 ml. to 47 ml. This resulted in a useful reduction in the volume of the air sample necessary for concentration measurements. A vacuum gauge (V.G.) was added to D_1 so that the rate of pumping in the system could be followed without the use of a mercury manometer. A tower (H) packed with ' Hopcalite ' (' Mercurysorb ') was used to remove any traces

* Part X: *J. Sci. Fd Agric.*, 1954, **5**, 536

† This work forms part of a thesis approved for the Ph.D. degree by the University of London

of mercury vapour which might have been present in the air entering the system from the laboratory.

Because of the greatly increased sensitivity of the detector the method of preparing mercury–air mixtures was slightly modified in the lower concentration range (below 0·5 mg./m.^{3}). A small subsidiary vessel (S.V.) of 75 ml. capacity was added in the position shown : this vessel could be filled to a known pressure with mercury-saturated air which could then be further diluted with more

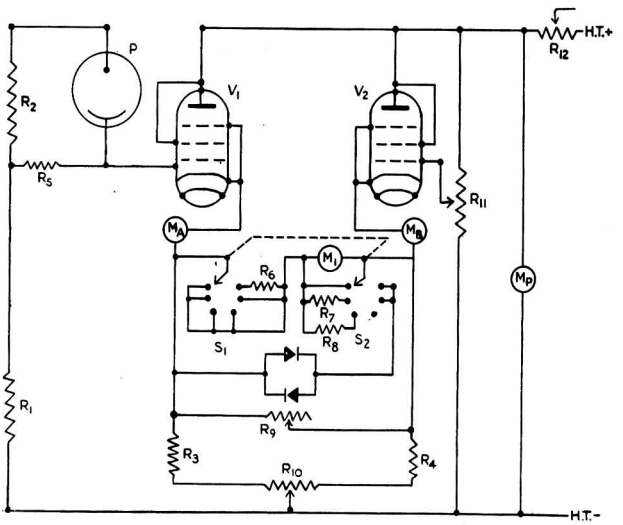

FIG. 1.—*Amplifier : circuit diagram*

Circuit values

R_1	470,000 Ω ± 20%, 0·25 watt	R_7	80 Ω ± 10%, 0·1 watt
R_2	2 Ω ± 20%, 1 watt	R_8	1000 Ω ± 10%, 0·1 watt
R_3, R_4	27,000 Ω ± 5%, 5 watt	R_9	1000 Ω, 3 watt : meter rheostat
R_5	10 Ω ± 20%, 0·25 watt	R_{10}	25,000 Ω, 3 watt : fine balance
R_6	220 Ω ± 10%, 0·1 watt	R_{11}, R_{12}	500,000 Ω, 3 watt : coarse balance

P Photoelectric cell, Cinema-Television Ltd. QVA38
V_1, V_2 Mazda SP61 (matched)
S_1, S_2 Two-wafer Yaxley six-position meter switch
M_P 300 μa. full scale deflection : 1 MΩ total resistance
M_A, M_B 5 μa. full scale deflection
M_1 100 μa. full scale deflection ; 1100 Ω resistance (indicator meter)

clean air in the mixing vessel. Concentrations as low as 2 μg./m.^{3} could be established with reasonable accuracy using this method, and the meter gave a detectable response to this concentration on the highest sensitivity range.

Another consequence of the increased sensitivity of the detector was that 50-cycle a.c. flicker from the ultra-violet lamp was picked up by the photocell and appeared on the indicator meter when the highest sensitivity range was being used. The use of a resonance cell (R.T.) as a source of ultra-violet radiation resulted in a considerable improvement in this respect. The evacuated quartz tube used as the resonance cell was 1½ in. in diameter and 3 in. long and contained a very small droplet of mercury. It was mounted close to the lamp and co-axially with the light-absorption tube (LA) in the position shown in Fig. 2. After the addition of the resonance cell it was found that the residual oscillations of the meter needle were barely perceptible on the highest sensitivity range. There was also a slight improvement in the overall sensitivity of the detector, presumably due to the fact that the light passing through the light-absorption tube to the photocell was now more nearly monochromatic 2537 Å radiation.

With the improvements that have been described, the lowest concentration of mercury vapour detectable under favourable conditions (low atmospheric humidity) was 2 μg./m.^{3} but in wet weather this limit was sometimes raised to 5 μg./m.^{3} due to slight instability of the amplifier caused by leakage currents. The actual weight of mercury in the tube LA at the lower

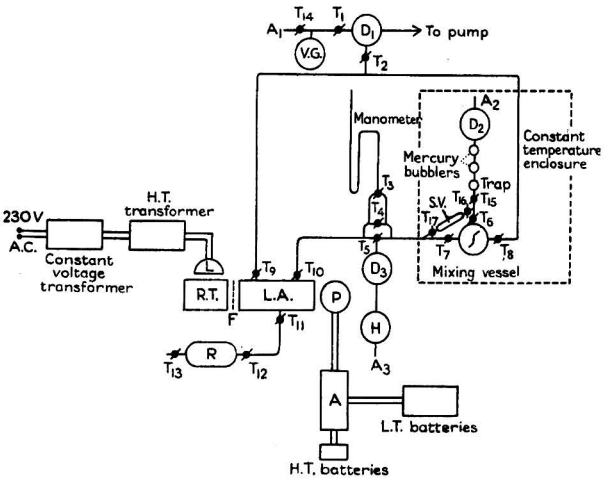

FIG. 2.—*Diagram of apparatus*

A	Amplifier	T_s	Three-way capillary tail-tap
D_1	Anhydrone drying tower		All other taps; two-way capillary
L	Lamp	A_1, A_2, A_3	Air inlets
R	Reaction vessel	D_2, D_3	Silica gel tower
H	'Hopcalite' tower	P	Photocell
V.G.	Vacuum gauge	R.T.	Resonance tube
T_s	Three-way capillary tap	S.V.	Subsidiary vessel

concentration was 10^{-10} g. The reproducibility of results was determined by the stability of calibration of the detector which was affected by the slight changes in the spectral emission of the lamp noticed in the original apparatus. In order to minimize calibratory errors, the lamp was run continuously and the apparatus recalibrated as soon as possible after each determination of an unknown concentration. It seems likely that the stability of lamp emission and hence of the detector as a whole could have been improved if precise control of the lamp temperature had been possible.

Sorption of mercury vapour on glass

With the original detector, capable of detecting mercury vapour at a concentration of 0·1 mg./m.^{3}, no sorption on the internal surfaces of the 1-litre glass sampling vessels could be detected. Similar experiments were carried out with the improved detector, the results of which are given in Table I. After these experiments, the sampling vessels were flushed with air to

Table I

Sorption of mercury vapour by Pyrex glass: temperature 25°

Sampling vessel No.	Time (days)	Initial concentration mg./m.^{3}	Final concentration mg./m.^{3}	Difference mg./m.^{3}	Change %
1	4	3·21	3·30	+ 0·09	+ 2·7
2	4	3·06	2·85	− 0·21	− 6·9
3	4	2·72	2·55	− 0·17	− 6·3
4	8	3·17	3·17	0·00	0·0
6	8	3·21	3·30	+ 0·09	+ 2·7
7	8	3·20	3·05	− 0·15	− 4·7

remove mercury vapour and then set aside full of clean air for seven days. After this time no mercury could be detected in air samples drawn from any of the vessels even using the highest sensitivity range of the detector (concentration less than 2 μg./m.^{3}). This indicated that the amount of mercury sorbed by the Pyrex glass vessels was very small and would not interfere in the subsequent experiments on the sorption of mercury vapour by wheat.

Sorption on wheat at different temperatures

The methods used for investigating sorption on wheat have already been described in an account of preliminary experiments.[1]

A further series of experiments was carried out with the improved detector, to cover a much wider range of temperatures (11° to 35°) using wheat of two different moisture contents (12% and 16%). The initial concentration was near 6·0 mg./m.^{3} and the time allowed for sorption was 24 hours. After this period the concentration remaining in each vessel was measured, and the vessel flushed with air until no mercury could be detected in the issuing gases, using the most sensitive range of the detector. The vessel was then set aside at the same constant temperature for a further 24 hours, when the concentration was again determined. In this way, a measure of the relative amount of mercury desorbed from the wheat at various temperatures (and, by difference, that firmly retained) was obtained.

The results of these experiments are shown in Table II. Sorption is expressed in terms of the Q_s/Q_f ratios used by Lubatti & Harrison[3] to bring fumigation experiments to a comparable basis. An increase in temperature over the range studied results in some increased sorption, but this increase is very small between 10 and 30°.

Table II

Sorption on wheat at different temperatures

Moisture content	Quantity sorbed/quantity free, Q_s/Q_f				
	Temperature °c				
%	10	15	20	25	30
12	0·3	0·3	0·2	0·6	0·5
16	2·0	4·0	3·6	2·0	4·0

Sorption on wheat of different moisture contents

The experiments described in the earlier paper have been extended, using the improved detector, to cover a range of moisture contents from 6% to 24% at a single temperature, 25°. The time allowed for sorption was again fixed at 24 hours, but after flushing with air at the end of this period, the wheat was left for a further eight days before the amount desorbed was measured.

The results of these experiments are shown in Table III. At moisture contents higher than

Table III

Sorption on wheat at different moisture contents and constant temperature of 25°

	(Quantity sorbed/quantity free)					
Moisture content %	6	8	9	12	16	18
Hg sorbed after 24 hours	0·5	0·4	0·6	0·8	2·0	4·5
Hg desorbed during airing for 8 days	0·4	0·2	0·2	0·1	0·1	0·0

18% virtually all the mercury in the sorption chamber was sorbed within 24 h. and the subsequent desorption was negligible. The important influence of moisture content on sorption is clearly shown, an increase of moisture content resulting in very much increased sorption between 14% and 18%: above this range all mercury appears to be irreversibly combined, while below it a certain amount is recoverable by airing.

The airing of wheat after prolonged contact with mercury vapour

In his work on mercury as a control for stored grain pests, Wright[4] reported that wheat that had been in contact with mercury vapour for several months showed no trace of contamination with the metal when examined spectroscopically. Adult *Calandra granaria* bred vigorously in the grain, and the wheat showed no reduction in germinative power. The experiments just described, however, show that a certain amount of mercury is recoverable, in the vapour phase, from wheat of low moisture content that has been in contact with the vapour, although most of the mercury appears to be irreversibly combined. Some experiments were carried out to find how long the airing process would have to be continued to remove all the recoverable mercury from samples of wheat that had been in contact with the saturated vapour for prolonged periods.

Methods of airing wheat samples.—The first sample of wheat examined had a moisture content between 15% and 16%. About 1 kg. of the wheat had been placed in a desiccator over a pool of purified mercury, but separated from it by an iron-wire gauze. The desiccator had been kept almost undisturbed for two years at room temperature. At the end of this time the mercury surface was found to be still relatively clean, and untarnished by the film generally noticed after a few days' exposure to ordinary laboratory air. This observation suggested that a saturation concentration was being maintained in the desiccator.

The wheat sample was thoroughly mixed and 100 g. were placed in a 1-litre reaction vessel, the joint greased, the taps closed and, after half an hour, a gas sample removed and analysed for mercury. A concentration of 1·5 mg./m.^{3} was present in the vessel. The vessel was flushed with air for half an hour, after which time the issuing gases contained less than 5 μg. of mercury/m.^{3}, set aside for two hours and another sample taken. The concentration had again risen to 1·5 mg./m.^{3}. The vessel was flushed with air until the mercury concentration in the issuing gases was again less than 5 μg./m.^{3}, and kept for a further two hours when the concentration had risen to 1·15 mg./m.^{3}.

After flushing once more with air, the vessel was placed in the constant-temperature enclosure at 25° for 24 hours when it was again sampled, flushed with air, and this procedure repeated every 24 hours. It was found after 40 days that the wheat was still evolving mercury but that the concentration building up in the vessel each day was decreasing slowly (Fig. 3).

Two further wheat samples, of moisture content 12% and 16% respectively, were also placed over pure mercury in desiccators at room temperature but for a shorter period of time, namely six weeks. 100 g. of each sample, and a further 100 g. of the original sample tested (exposure time two years), were placed in separate shallow paper trays and allowed to air in a laboratory at about 16°. Airing conditions obtained in this way were unaffected by the diffusion rate through the inter-granular spaces of the wheat, since the trays were large enough for the wheat to be spread out only one grain deep, with few adjacent grains touching. After 24 hours, the samples were placed in cleaned reaction vessels with the taps closed and kept for 24 hours in the constant-temperature enclosure before measuring the gaseous concentration. They were then aired in the trays for 24 hours and the procedure repeated until the concentration had fallen below 2 μg./m.^{3}. In the case of the two samples that had been in contact with saturated mercury vapour for only six weeks, this occurred in six days; but the third sample (exposure time two years) was still giving detectable amounts of mercury vapour after 14 days.

Another experiment with the same three samples was designed to find the maximum concentrations that would be reached in a vessel if the mercury were not removed from the air above the wheat (by flushing with air) as in the previous experiments. 100 g. of each of the three samples of wheat were allowed to air in paper trays for 24 hours, placed in separate reaction vessels (of 1 litre capacity) and the gaseous concentration measured every 24 hours, without subsequently flushing the vessels with clean air. Two samples were taken on each occasion (total volume 94 ml.) and clean air was then allowed to enter the vessel to restore it to atmospheric pressure.

In this type of experiment the gaseous concentration of mercury might be expected to alter initially in one of three ways: (i) The concentration might fall off at a rate that could be calculated from the amount of dilution occurring after each pair of samples had been taken. This would indicate either that mercury was being evolved from, and absorbed by, the wheat at equal rates, or that any evolution of mercury was too slow to be detected. (ii) The concentration might not fall off as quickly as the calculated rate, showing that mercury was being evolved from the wheat faster than it was being absorbed. (iii) The concentration might fall off more quickly than the calculated rate, showing that mercury was being re-absorbed by the wheat faster than it was being evolved.

In a sample of the wheat that had been in contact with mercury vapour for two years it had been expected that the concentration would behave as in (ii) above. In fact, it was found in all three cases that the concentration fell off more rapidly than the rate calculated from the first measured concentration, although the difference was much greater in the case of the two samples that had only been in contact with the saturated vapour for six weeks. Fig. 4 shows the expected and actual rates of fall in concentration for each sample.

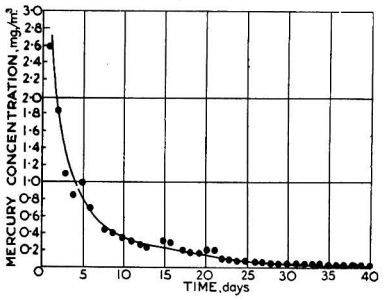

FIG. 3.—*Desorption of mercury vapour from wheat after two years' exposure to the fumigant*

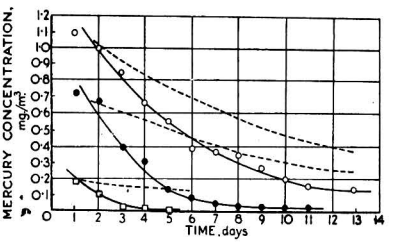

FIG. 4.—*Desorption of mercury vapour from wheat repeatedly flushed with air*

○ 15–16% moisture content : 2 years' exposure
● 12% ,, ,, 6 weeks' ,,
□ 16% ,, ,, 6 ,, ,,
— — — — theoretical desorption curves.

The diffusion of mercury vapour through grain

Since the work on the toxicity of mercury vapour to the eggs of *C. granaria*[5] had provided an estimate of the concentration–time product necessary to prevent hatching, it appeared that study of the diffusion of mercury vapour through wheat would give an indication of the effectiveness of the vapour as a protection for stored grain products.

Richards,[5] Nasir,[7] Krishnamurti & Appanna[8] and Puri & Bharihoki (unpublished) have investigated the diffusion of mercury vapour through grains. All these workers used the viability of insect eggs as the criterion of the presence of mercury vapour. Richards found that at 25°, mercury vapour would penetrate at least 30 cm. of grain in two days, and that at 12° the vapour would penetrate at least 90 cm. of grain in seven days. It appears from the work of Nasir and of Puri & Bharihoki that in their experiments mercury vapour diffused a distance of at least 36 in. through sorghum grains.

A spun cement–asbestos rain-water pipe, 175 cm. long, 7·5 cm. internal diameter, with a joining collar at one end was obtained. Seven half-inch-diameter holes were drilled through the wall of the pipe at 25-cm. intervals, measured from the base of the collar, and capillary glass taps were inserted in each hole through rubber bungs. The ends of the capillary tubing inside the pipe were covered with small pieces of muslin held in place with thin rubber bands. A piece of 14-mesh plastic gauze was cemented to a Tufnol ring and the ring fixed inside the pipe, next to the collar, by means of set-screws through the wall of the pipe. The pipe was placed on end, collar down, filled with English wheat of moisture content 16%, and a second disc of plastic gauze attached to a Tufnol ring pushed inside the open end of the pipe to hold the wheat in place. The pipe was then placed horizontally with the capillary sampling tubes vertical, the taps closed and a small open Perspex box containing purified mercury placed inside the collar. A disc of Perspex was then cemented to the end of the collar and the pipe kept at laboratory temperature.

After 24 hours, samples were taken directly into the light-absorption tube of the improved detector, one through each capillary sampling point, the first sample being taken at the point remote from the mercury vapour source. The procedure was repeated after a further 24 hours and then at intervals for a total period of 21 days. The Perspex disc was then removed from the end of the collar, the box of mercury withdrawn, the disc replaced and the tube sampled until the concentrations fell below the limit of sensitivity of the detector. During this experiment the laboratory temperature was observed to vary between 14° and 16°. Since the moisture content of the wheat was 16% the conditions of the experiment approximated to those found in grain stores in temperate climates. One further experiment was therefore carried out to investigate diffusion under tropical or sub-tropical conditions.

The wheat in the pipe was replaced by a sample of moisture content 11·4% and the experiment repeated. This time, however, the pipe was kept in a constant-temperature room at 25° and 65% relative humidity between sampling. The concentrations obtained along the pipe after various times under the two sets of conditions used are expressed graphically in Figs. 5 and 6. A steady state was reached quite rapidly, the amount of mercury diffusing along the pipe balancing the amount lost due to (i) sorption by the wheat, (ii) sorption by the walls of the pipe

and (iii) diffusion through the walls of the pipe. It was considered unlikely that factors (ii) and (iii) would have any effect on the experiment.

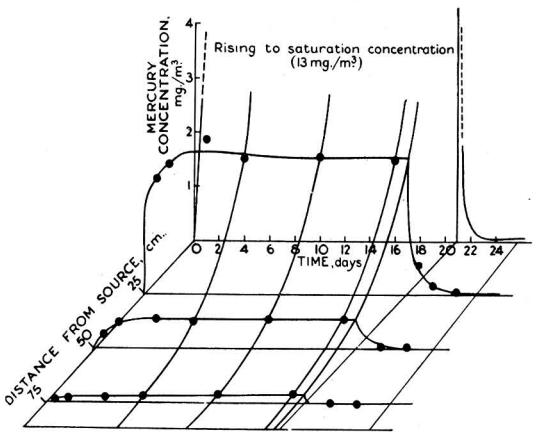

FIG. 5.—*Diffusion of mercury vapour through wheat under temperate conditions*

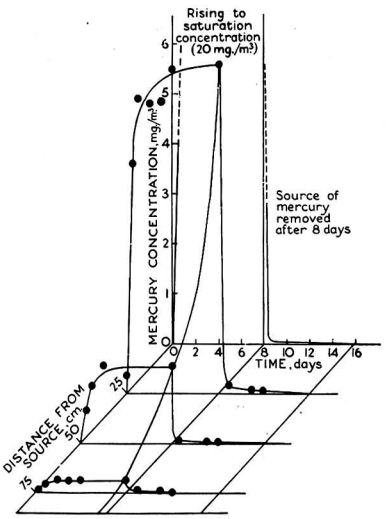

FIG. 6.—*Diffusion of mercury vapour through wheat under tropical conditions*

Discussion

It can be seen from Table III that the sorption of mercury vapour by wheat is affected by changes in moisture content to a much greater extent than changes in temperature (Table II) over the range of values found in practical wheat storage. The preliminary experiments on the effect of temperature[1] indicated that an increase of temperature over the range 20–30° results in an increased rate of sorption. The later experiments scarcely confirmed this view, and showed that the magnitude of the effect was not as great as had been first supposed. The experiments on the effect of moisture content showed that an increase in this factor results in an increase in the rate of sorption of mercury vapour, which is apparently firmly retained by wetter wheat. At low moisture contents it appears that most of the mercury is still firmly retained, but that there is a small proportion of the vapour physically sorbed which is more readily recoverable (e.g., by

airing). Lubatti & Smith[9] have shown that this type of behaviour is characteristic of those fumigants which react strongly with the seed tissues. Such behaviour is illustrated by Fig. 7 of the earlier paper.[1] A steady state can be seen from this figure to continue for at least eight days in the case of mercury in contact with wheat. Lubatti & Smith have shown that for methyl bromide in contact with onion seed the steady state continues for at least 12 days.[9]

It remains to decide how long this absorption of mercury continues, and it is here that the significance of the results obtained, both from airing wheat after prolonged contact with the saturated vapour and the diffusion of mercury through a column of wheat, becomes apparent. The experiments on diffusion through wheat showed that after 21 days a steady state was still obtained in the wheat column, indicating that the rate of uptake of mercury by the wheat was not decreasing even after this time.

The experiments on airing indicated that after two years mercury is still reacting with the wheat tissues.

Although no measurement of residues in wheat has been possible, an estimate of the order of magnitude of the amount of mercury combining with wheat during prolonged contact can be attempted, from the experiment in which a sample of wheat, moisture content 16%, had been in contact with saturated mercury vapour at room temperature for two years. This wheat was allowed to stand in a closed vessel; the concentration in the vessel was then measured at intervals, and the mercury vapour removed from the gas space by flushing with air immediately after sampling. The first three samples, taken after $\frac{1}{2}$, $2\frac{1}{2}$ and $4\frac{1}{2}$ h., contained 1·5, 1·5 and 1·15 mg. of mercury per m.^{3}, respectively, and the total weight of mercury recovered from the 100-g. wheat sample after eight days was 13·6 μg. In the experiments on the effect of moisture content, 100 g. of wheat that had sorbed 4·25 μg. of mercury gave up 0·01 μg. after eight days, 4·24 μg. being firmly retained. From these figures the amount of mercury combined after two years would be 5·8 mg./100 g. of wheat. The experimental results suggest that the sorption of mercury vapour by wheat is predominantly chemical in nature, most of the mercury combining irreversibly with the wheat. The most likely fate of this non-recoverable mercury is that it combines with the sulphur of the proteins of the wheat. The rate of reaction is accelerated at high moisture contents, presumably due to increased permeability of the seed coat and hydrolysis of the protein facilitating more rapid reaction. At low moisture contents there is evidence that some physical sorption is taking place, since more mercury is recoverable by airing. This is probably due to more capillaries becoming available, in the seed structure, into which mercury can diffuse, but which at high moisture contents are filled with water.

The experiments on the sorption of mercury vapour by, and diffusion through, grain show that mercury is not by any means an ideal fumigant since it combines strongly with the wheat tissues. In the experiments carried out using columns of grain, a steady state was reached quite rapidly, the amount of mercury diffusing along the column balancing that lost by sorption. Sorption appears to continue for a very long time. With a good fumigant which does not react strongly with the wheat tissues (e.g., methyl bromide) there would only be a small concentration-gradient along the wheat column, and the concentration would only fall off slowly when the source of fumigant was removed. This is far from the case with mercury vapour. The fact that mercury combines strongly with wheat is to some extent offset by the fact that it is extremely toxic, in low concentrations, to insect eggs.

The results of this work on diffusion confirm those of other workers.[5–7] Richards[6] reported that at 25° mercury was effective as an ovicide at 30 cm. from the source after two days, and at 90 cm. after seven days at 12°. In the present experiments the concentrations obtained after eight days at 25° were 5 mg./m.^{3} at 30 cm. and 0·25 mg./m.^{3} at 90 cm. from the source. The concentration–time products achieved after five days at 30 cm. and 90 cm. were thus 600 mg. h./m.^{3} and 30 mg. h./m.^{3}, respectively.

Studies on the toxicity of mercury vapour to various strains of *C. granaria* have shown that a wide variation in the resistance of 'wild' cultures is possible.[5] It seems likely from the figures quoted above that the insects used by Richards must have been of the same order of resistance as the least resistant 'wild' cultures examined by Blackith & Gorringe.[5]

Because of the wide variation of resistance among normal populations of grain weevils, prediction of the radius of toxic concentrations of mercury vapour diffusing from sources placed

in the grain is difficult. Control of *Calandra* eggs, assumed to be from a strain that had not become resistant to mercury vapour, would be achieved if no part of the mass of grain were further than two feet from a source of vapour. This conclusion may need modification if air movements occur in the intergranular spaces. In spite of the great dangers which attend the high and insidious toxicity of mercury to man, there seems little risk of dangerous concentrations being built up in the free spaces of grain stores, the normal leaky character of which should provide sufficient ventilation. The residues of combined mercury in the grain seem likely to occur in the form of the insoluble and unmetabolizable sulphur compounds.

Acknowledgment

This work has been supported by a grant from the Agricultural Research Council, which is gratefully acknowledged.

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Received 11 May, 1955

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FUMIGATION OF AGRICULTURAL PRODUCTS. XII.*— Sorption of Methyl Bromide on Groundnuts†

By H. M. B. SOMADE

Groundnuts, both undecorticated and separated into husk, cotyledon and germ, have been fumigated with methyl bromide. The sorption of the fumigant increases in all cases with the moisture content. Sorption is disproportionately greater as dosage increases, for cotyledons and germ, but not for husks. Over a wide range of relative humidities, the equilibrium moisture content of the husk is greater than, and that of the cotyledons less than, the moisture content of the germ.

Field trials in England and in Nigeria have confirmed that under normal conditions groundnuts are unharmed by methyl bromide fumigation. Fungal attack is suppressed and early development mildly stimulated by the treatment.

High doses of methyl bromide will impair the germination of groundnuts of too high a moisture content. The tip of the radicle is destroyed, but there is no evidence of combination of the gas with any major constituent of the seed.

Groundnuts, of which some half million tons are grown annually in northern Nigeria, were perforce stored in the open until recently, for periods of up to three years, because of the inadequate facilities for transport to the ports. Covered only with tarpaulins during the rainy seasons, the pyramids of bagged nuts are liable to severe insect infestation, which may produce collapse of a pyramid if it is left untreated. Moreover, perforation of the groundnuts by insects

* Part XI: Preceding paper
† Part of this work formed part of a thesis approved by the University of London for the M.Sc. degree

sets up lipolytic enzyme action which increases the free fatty acid content, often to the point where the oil is fit only for soap-making.

The most severe damage is caused by the larva of the beetle *Trogoderma granarium* Everts. These larvae are notoriously resistant to contact insecticides, and, in practice, fumigation of the pyramids under the tarpaulins with methyl bromide has proved the only satisfactory control measure yet devised. The process is as yet somewhat empirical, little information being available about the suitability of the fumigated nuts for seed purposes, or about the sorption of methyl bromide on the nuts at different moisture contents. Such information is needed before the most economical dose of fumigant can be assessed.

Experimental

The groundnuts used in this work were mainly samples from the 1952/53 Nigerian crop, a small sample of the 1951/52 crop being included for comparison.

Lubatti & Smith[1] have pointed out that with the larger seeds, such as groundnuts, bigger samples are needed to give reproducible results in sorption experiments than with smaller seeds. The samples used in this work, usually 200-g. lots of undecorticated nuts, were treated in a 5-litre aspirator with suitable attachments. The fumigation of the less bulky samples of decorticated nuts, and of germ, was done in a 1-litre glass (' Turtle ') chamber. Known quantities of methyl bromide were introduced in ampoules fractured within the above containers. Samples of air were withdrawn from the containers by the method of Page,[2] the methyl bromide catalytically burnt to bromine,[3] and the bromine determined by Wade's modification of the potentiometric method.[4]

Moisture contents of the undecorticated nuts were estimated by an oven-drying technique described by Hoffpauir[5] as suitable for oily seeds. In this method seeds are heated at 130° for five hours, and Pickett[6] has observed that the proteins of groundnuts withstand 140° without decomposition. However, the charred appearance of the dried nuts suggested that decomposition was taking place and the moisture in the cotyledons was estimated by drying at 105°. The delicate germ tissues were subjected to moisture content determinations by the acetyl chloride–pyridine method[7] to avoid artefacts caused by heating the germ.

The intergranular space in the different fractions was estimated by the method of Jones,[8] so that the amounts of sorbed and free methyl bromide in the fumigation chambers could be calculated.

Samples of 200 g. of undecorticated groundnuts conditioned to moisture contents of 5·05%, 10·11% and 14·55%, respectively, were placed in the chambers. Two concentrations of methyl bromide were applied in each of two series of fumigations, one at 10 mg. per litre, the other at 30 mg. per litre. All the experiments were done at 30° and for 24-h. exposure periods.

I. Assessment of germination

Experiments in England.—The fumigated nuts were aired, and then decorticated by hand before division into two batches. One batch was planted in light gravelly soil simulating Nigerian conditions. The other set were germinated in sand in a constant-temperature room, transplanted to pots and allowed to develop in a glasshouse. The plants in the open were arranged in randomized blocks with ten plants in a plot. Each of the four blocks was a complete replicate of the experiment, and contained, in addition to the treated nuts, unfumigated samples from the 1951/52 and 1952/53 crops, the latter at two moisture contents (5 and 14%). Thirty nuts in each group were sown for the glasshouse experiment; most of the nuts had germinated within three weeks and were potted and transferred under glass.

Experiments in Nigeria.—Since England is well outside the tropical belt within which groundnuts will grow and ripen normally, a further experiment on similar lines was laid out at Kano, northern Nigeria, from June to October, 1955. Groundnuts of the 1953/54 crop were planted as in England, after the appropriate fumigation treatment. As in the English experiment, a row of plants from an earlier crop was included in each replicate: in these experiments nuts of the 1952/53 Nigerian crop, of 5% moisture content, were fumigated at 30 mg./l. for 24 h. One of the blocks was attacked by termites, which seemed to attack the rows of plants impartially.

The groundnuts planted at Kano matured and ripened normally, so that a direct assessment of yield in terms of the weight of shelled nuts was possible. Germination records and an assessment of the time of first flowering were also kept.

Results

Experiments in England

Several different measures are available to estimate the response of seeds to damage by a fumigant. Under English conditions, although most of the groundnut plants flowered, only two nuts were formed, so that the overall yield could not be used as a measure of damage. Two other measures were used, one was the percentage germination of each batch, the other the dry weights of the mature plants. Records of the times at which the plants emerged from the soil, and of the flowering time, were also kept.

Germination counts were made both on the seeds planted in the open and those planted under glass in England. The results obtained are shown in Table I. The most important and

Table I

Germination of groundnuts fumigated with methyl bromide

Grown in		England Glasshouse in sand	England Open air in soil	Nigeria Open air
Moisture content %	Dosage mg./l.	% (of 30)	% (of 40)	% (of 40)
5	controls	93	48	95
10	controls	80	45	48
5	10	87	50	98
10	10	97	58	70
14	10	33	48	0
5	30	97	48	100
10	30	90	60	18
14	30	0	0	0

decisive conclusion is that germination of dry nuts is unaffected by methyl bromide fumigation up to the highest concentration examined, but that the fumigation of nuts of high moisture content with high doses of methyl bromide completely inhibits germination. Comparison of Table I and Fig. 2 leaves little doubt that the relatively narrow range of conditions over which damage becomes severe is associated not only with the sorption of methyl bromide by the nuts but also with their moisture content. This quantity seems unusually critical, in that nuts of 5% moisture content can sorb 377 p.p.m. of fumigant without apparent damage, but when the moisture content is raised to 14%, as little as 131 p.p.m. of sorbed methyl bromide reduces the germination by some 60%.

The results of the germination counts in the glasshouse experiments closely followed those obtained in the open. One interesting and unexpected result could be observed with the seeds planted in sand for transference to the glasshouse. Even when seeds had been killed by the fumigation, they remained free from obvious fungal attack, whereas unfumigated seeds, including viable ones, were often attacked by fungi. Moreover, as is apparent from Table I, fumigated seeds of low moisture content give slightly higher germination in the field than the unfumigated seeds, and the results of the experiments in sand confirm this tendency. These germination results which suggest enhanced viability after treatment are not statistically significant in themselves, but the number of nuts failing to germinate is normally small. An adequate trial of the suggested effect would need to be extensive. The direct observations of appearance seem, however, decisively to demonstrate that methyl bromide prevents visible fungal development in all treated nuts, and stimulates development of the embryo and subsequent growth of the seedling. This stimulation is shown most clearly by the lengthening of the plumule before the opening of the first pair of leaves. The increased rate of development persists, and there was a clear gap between the date of flowering of all the plants from fumigated nuts and the corresponding date for

plants from untreated nuts of either the 1951/52 or 1952/53 crops, both of which flowered 10 days after the treated plants. A similar interval was also noted in the plants grown in the glasshouse.

When it became clear that for obvious climatic reasons only two of the many plants grown in the open were going to set fruit, all the plants were dug up, the roots washed free from soil, and the plants dried in a steam oven. The total dry weight of the plants in each group (see Table II) followed closely the number germinating in that group. An analysis of covariance revealed that no differences between the groups of nuts could be discriminated by means of their total dry weights that was not already discernible from the proportions germinating. This conclusion suggests that the dry weight of a plant at maturity is independent of fumigation treatments applied to the nut, and that the stimulation effected by methyl bromide accelerates but does not enhance development.

Experiments in Nigeria

The germination counts were subjected to an analysis of covariance similar to that applied to the English experiments. From this analysis for which, as is usual, the percentages were transformed into angles, we find that the dry weights of shelled nuts from each plot depend only on the number of seeds which germinate. The fumigation treatments influence the yield of nuts,

Table II

Total dry weight of plants and of shelled nuts from plots of fumigated groundnuts

Grown in		England (g. of plant material)	Nigeria (g. of shelled groundnuts)
Moisture content %	Dosage mg./l.		
5	controls	15·2	1247·8
10	controls	26·9	958·0
5	10	31·4	1589·1
10	10	35·3	582·3
14	10	22·5	0·0
5	30	36·7	1933·5
10	30	33·8	114·4
14	30	0·0	0·0

therefore, only in so far as the germination is modified by the treatments. This influence of the treatments on germination was thought worthy of further statistical examination. The mean transformed germination counts for each treatment were ranked in order of magnitude, and the least significant interval between adjacent means was computed.[9] All the nuts fumigated at 5% moisture content germinated uniformly well, and fall into the same group as the unfumigated nuts of that moisture content. Even the older nuts of the 1952/53 crop given the highest dosage of methyl bromide (30 mg./l.) germinated as well as the controls of their moisture content group (5%). The overriding importance of moisture content is disclosed by the fact that the control (unfumigated) nuts of 10% moisture content germinated significantly less well than lightly fumigated nuts of this moisture content, and both these categories were inferior to the drier nuts. The heavily fumigated nuts of 10% moisture content were again inferior to the control or to lightly fumigated nuts of this moisture content. None of the nuts of 14% moisture content germinated.

In these experiments all the fumigated nuts gave rise to plants which flowered four days earlier than did those from unfumigated nuts, irrespective of their moisture content. The nuts given the highest dose at the low (5%) moisture content were the only ones for which complete germination was recorded in the field. Difficult as it is to provide adequate statistical assessment of the general health and growth of seedling plants, there remains little doubt of the reality of the stimulation to early growth afforded by methyl bromide fumigation.

II. *Sorption by constituents of the groundnut*

The undecorticated groundnut has a hilum through which fumigants may penetrate, in addition to the diffusion which may occur through the tissues of the pericarp or husk. The decorticated nut itself is divisible into the germ and the cotyledons, which contain about 50% of oil and a remainder which is mainly protein material.

Before the sorption of methyl bromide on these constituents could be estimated, their moisture contents when in equilibrium with one another and with a definite relative humidity had to be determined. These equilibria were obtained by storing the separated fractions in desiccators over saturated salt solutions of known vapour pressure. The results are shown in Fig. 1, and confirm the observations of Karon & Hillery[10] that, when in equilibrium, the moisture contents of the constituents differ substantially.

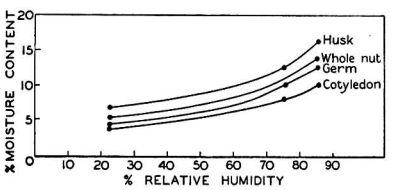

FIG. 1.—*Moisture contents of seed constituents when in equilibrium with known R.H.*

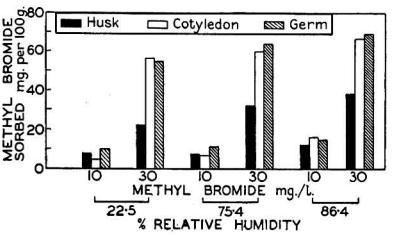

FIG. 2.—*Sorption of methyl bromide on seed constituents*

The constituents were then fumigated with methyl bromide at the two levels used for the germination experiments, namely 10 and 30 mg. per litre. Fig. 2 discloses that both the cotyledon and germ sorb similar amounts of methyl bromide, which are higher than that sorbed by the husk. An important result is that when the cotyledon and germ are treated with methyl bromide at 30 mg. per litre they sorb more than three times as much fumigant as at 10 mg. per litre, whereas the husk sorbs methyl bromide in amounts proportional to the concentration of fumigant.

The oiliness of the nuts prevented their being ground satisfactorily in an enclosed mill, even when an absorbent diluent was added before grinding. This inability to grind the seed in an enclosed mill precluded the use of Lubatti's wet aeration method for the estimation of reversibly held fumigant[11] and, hence, by difference, the firmly held methyl bromide.

Sorption in groundnut oil

At 30°, water and groundnut oil absorb similar amounts of methyl bromide (2·2 and 2·5 g. per litre respectively). However, when oil extracted from fumigated nuts was tested for halide by Lassaigne's test the result was negative. About 0·45 mg. of hexachloroethane was dissolved in 5 ml. oil and 0·5 ml. of this solution gave a positive Lassaigne test, demonstrating the validity of the method at the halide concentrations expected. This result suggests that the ethylenic bond in the major constituents of groundnut oil does not react with methyl bromide under the mild conditions of a fumigation.

Reaction of methyl bromide with germinal steroids

As an example of the type of steroids which occur in the germ, ergosteryl acetate (1 g.) was dissolved in carbon tetrachloride and liquid methyl bromide added in excess. After the mixture had been set aside at room temperature, the volatile liquids were removed under reduced pressure, and the residue recrystallized from light petroleum. The crystals so recovered had the same melting point as ergosteryl acetate (170–172°) and gave a mixed melting point of 171–173°, showing that no reaction had occurred.

Discussion

The work reported in this paper shows that the successful treatment of groundnuts with methyl bromide depends on the limitation of the moisture content of seed. The nuts should not be allowed to exceed 5% moisture content or the germination may be seriously impaired. Nuts

having a higher moisture content than 10% have poor keeping qualities under tropical conditions, and would be purchased for storage only with reluctance, so that there is a strong inducement to growers to keep the nuts dry.

In practical fumigations, careful control of dosage and circulation of fumigant are desirable to keep the concentration to about 30 mg. per litre at any part of the bulk of nuts. The demonstration that sorption increases more rapidly than does the dosage emphasizes the importance of controlled dosage. However, with nuts of low moisture content, there is a wider range of doses of methyl bromide which are tolerated by the seeds, and indeed there is some evidence that such treatments may help any seeds that may be planted to get away quickly while the soil is still moist after the rains. The gas may have destroyed harmful fungi and bacteria. Rehm[12] has demonstrated a similar stimulation with sterilized sugar beet seed. Certainly there is a striking absence of fungal attack on all fumigated seeds, compared with the incidence in the controls.

The action of methyl bromide is thus twofold. At any given moisture content a sufficiently high dose will kill the seed. The higher the moisture content the lower is this critical dosage, but the keeping qualities of groundnuts deteriorate so rapidly with increasing moisture content that even unfumigated nuts will die before germinating if of more than 10% moisture content. The influence of moisture content coupled with fumigation was more clearly defined in the Nigerian experiments, doubtless because the shade temperatures were much higher than in England (71–94·5° F).

At concentrations lower than that critical for a particular moisture content and period of fumigation, a slight but definite stimulating action has been observed both in England and in Nigeria. This phenomenon is shown most clearly for the Nigerian field trials with nuts of 10% moisture content, where the lightly fumigated nuts germinated significantly better, and the heavily fumigated nuts significantly worse, than did unfumigated nuts. No range of doses has been found which damages the seed without killing it, a result consistent with that of Lubatti & Blackith[13] who fumigated potatoes with methyl bromide. Although more extensive trials might disclose such a range, as happened in the potato experiments,[14] the economic importance of effects detectable only with large-scale trials is usually slight.

At doses sufficiently high to kill the seed, damage to the radicle was noted. The growing tip was discoloured for the terminal 2 mm. and the whole radicle ultimately shrivelled. The apices of growing shoots seem likely to be the part most sensitive to methyl bromide damage, as has been found with sprouting potato tubers.[13] There is no evidence that methyl bromide reacts with groundnut oil, or with the steroids of the germ. The gas will react with sulphur-containing protein materials and there is a substantial protein component of the cotyledons, but only a small fraction (about 2%) of these proteins are likely to contain sulphur. Some nitrogen linkages in un-ionized basic groups are also sensitive to methylation by methyl bromide in solution but again their contribution to the total sorption must be small.[15] Clegg & Lewis[16] were unable to detect a loss of vitamins of the B-group in groundnuts fumigated with methyl bromide.

So far as can be judged, no adverse effect on germination, stand, or yield is produced when groundnuts are fumigated with methyl bromide under the conditions normally found in practice.

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Received 11 May, 1955

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

DECEMBER, 1955

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

INDEX OF AUTHORS' NAMES

ERRATUM

J.S.F.A. Abstracts, 1955, ii

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* from bottom.

Subject Index, 1955, i, p. 103

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

DECEMBER, 1955

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Relation of certain loessial soils of Northeastern Kansas to the texture of the underlying loess. R. M. Hanna and O. W. Bidwell (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 354—359).—Textural and other characteristics of profiles on a 24-mile traverse in Northeastern Kansas, starting from the Missouri river valley and running perpendicularly to the river, are reported. The decreasing textural size of the loess and depth of loess with increasing distance from the river indicate that the river was the source of most of the loess deposits in this region. A. H. CORNFIELD.

Profile characteristics of some loess-derived soils and soil aëration. R. V. Ruhe, R. C. Prill and F. R. Riecken (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 345—347).—Characteristics of the soils are described and discussed, particularly in relation to the distribution of deoxidised and mottled zones formed through poor drainage and aëration. A. H. CORNFIELD.

Characterisation of some halomorphic soils and their normal associates in the Yakima Valley. J. U. Anderson (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 328—333).—The physical and chemical characteristics of a brown, a solonetz, and two solonchak soils, their geographical distribution, and their relationship to the geology of the Yakima Valley are reported. A. H. CORNFIELD.

Physical and mineralogical properties of related Minnesota prairie soils. H. E. Arneman and P. R. McMiller (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 348—351).—The characteristics of three soils of a catena developed on calcareous glacial till of Wisconsin age (Mankato substage) are reported. A. H. CORNFIELD.

Soils of the Dijon region. III. Texture. S. Mériaux (*Ann. Agron.*, 1954, **5**, 985—993).—The texture of soils of the region is characterised by both continuous wt. % and frequency distribution curves for particles from $<0{\cdot}8$ up to 2000μ. diameter. A. H. CORNFIELD.

Theory of capillary flow. I. Practical implications. E. E. Miller and R. D. Miller. **II. Experimental information.** R. D. Miller and E. E. Miller (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 267—271, 271—275).—I. The qual. explanation and interpretation of a form of capillary flow theory is presented.

II. Published experiments relating to capillary flow are examined for data to test the capillary flow theory. A. H. CORNFIELD.

Effects of long-term soil treatments on some physical properties of several Illinois soils. J. R. Gingrich and R. S. Stauffer (*Proc. Soil. Sci. Soc. Amer.*, 1955, **19**, 257—260).—The effects of three different fertiliser treatments [(*a*) no fertiliser, (*b*) manure + $CaCO_3$ + rock phosphate, (*c*) crop residues + $CaCO_3$ + rock phosphate + K] applied for 40 years to six cropped Illinois soils on water permeability, % of moisture at 0·33 and 15 atm. tension, bulk density, non-capillary pore space and max. available moisture of the soils were studied. Significant differences in values due to treatment were obtained in only a few cases. Usually soils under treatments (*a*) and (*c*) had the highest water permeability, whilst some of the soils under treatment (*c*) had a greater bulk density than did those under the other treatments. There were no consistent differences in any of the measurements between treatments (*b*) and (*c*). A. H. CORNFIELD.

Determination of soil compactibility. R. R. Bruce (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 253—257).—A simple impact type soil compacter, which requires not more than 800 g. of soil and 1 hr. of operation to yield bulk density–moisture curves for measuring soil compactibility, is described. Results obtained clearly indicated differences in compactibility due to soil treatment. Soil from plots which had been under continuous maize, with no fertiliser treatment, for a no. of years had a higher max. bulk density and a lower moisture content at max. bulk density, than did soil from a maize–oats–meadow rotation receiving balanced fertiliser. Compactibility values correlated with water-stable aggregation data and % of moisture retained at both 0·33 and 15 atm. tension. A. H. CORNFIELD.

Soil properties related to forest cover type and productivity on the Lee Forest, Snohomish County, Washington. F. F. Forristall and S. P. Gessel (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 384—389).—Physical and chemical characteristics of forest soils from five sites representing different species and site classes are presented. Only five characteristics appeared to be related to productivity, these being effective soil depth, bulk density, moisture, cation exchange capacity and total N content. The lower limit of the effective rooting depth generally corresponded with a marked increase in bulk density of the soil. The roots of different species varied somewhat in their ability to grow in areas of high bulk density. In general, the most productive soils were those with no hardpan or where the region of high bulk density was deep in the profile. Except for one plot, soil moisture deficiency was not a limiting factor. Excess soil moisture associated with poor internal drainage was a major factor in determining forest type. High productivity was associated with high soil N and base exchange capacity. A. H. CORNFIELD.

Soil moisture regime in some forest and non-forest sites in Northern Wisconsin. J. L. Thames, J. H. Stoeckeler and R. Tobiaski (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 381—384).—In a comparison of the moisture regime through the season between a forested silt loam and adjacent soil under timothy it was found that there was less available water, particularly towards the end of the year, in the forested area. The bulk density of the soil under timothy was 25—32% greater than that under forest. A sandy forested site having a permanent water table at about 2—3·5 ft. had higher available moisture throughout the season, and particularly early in the season, than did a comparable soil having a water table at 4·5—6 ft. The site index for aspen was greater in the area with the shallow water table, whilst that for jack pine was similar in both areas. The higher available moisture in the area with the shallow water table probably accounted for the invasion of this area by black spruce and tamarack. A. H. CORNFIELD.

Site factors affecting the success of reforestation and afforestation in the Willamette Valley foothills. C. T. Youngberg (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 368—372).—Profile characteristics, nutrient status, and physical properties, particularly moisture relations, of areas where reforestation and afforestation have been attempted are reported. The range of available moisture (water held at low tension) was relatively narrow for the four soils investigated. Even in seasons of above-normal rainfall soil moisture in the root zone reached the difficultly available range early in the season. This probably accounted for the poor survival of tree seedlings. A. H. CORNFIELD.

Effect of tractor logging on physical properties of some forest soils in Southwestern Washington. E. C. Steinbrenner and S. P. Gessel (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 372—376).—Some physical properties of soils from undisturbed forest, tractor skid roads, and the cutover area (that between the skid roads) of an area having heavy-textured soils is reported. Soil from the tractor skid road showed, on an average, 93% loss of permeability, 15% increase in bulk density, and 53% loss of macropore space in comparison with undisturbed soil. Soil from the cutover area also showed decreases in permeability rate and pore space and increase in bulk density. The skid roads covered an average of 26% of the logged area. A. H. CORNFIELD.

Effects of freezing and thawing, and wetting and drying on aggregation of soils treated with organic chemicals. W. O. Willis (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 263—267).—The effects of varying no. of cycles of freezing and thawing or of wetting and drying on water-stable aggregation of soils treated with 0·1% HPAN (hydrolysed polyacrylonitrile, an aggregate stabiliser), 0·2% SC-50 [a water-sol. Na methyl siliconate, $CH_3{\cdot}Si(OH)_2{\cdot}ONa$, which has a water-proofing effect on soil], or 0·0013% PR-51 (a water-sol. alkylated aromatic sulphonate, which increases water penetration in soil) was studied. The freezing–thawing or wetting–drying treatments caused a general decrease in aggregate stability in both treated and untreated soils. Soils treated with HPAN or SC-50 had higher aggregation than did control soils irrespective of the no. of cycles of freezing–thawing or wetting–drying. In general, the greatest breakdown in aggregates due to freezing–thawing for both treated and untreated soils occurred during the first cycle. The freezing–thawing treatments usually had a greater effect in causing aggregate breakdown than did the wetting–drying treatments. The aggregation in a Lagonda silt loam soil was increased by ten cycles of wetting and drying. Results obtained with soils treated with PR-51 were no different from those obtained with control soils. A. H. CORNFIELD.

Use of gas desorption isotherms and high pressure porosimeter in determination of pore structure of soil aggregates. D. R. Bianco (*Dissert. Abstr.*, 1955, **15**, 1155).—The porous structures of the widely differing soils, and of soil fractions from cultivated and virgin plots, were studied by (*a*) N_2 gas adsorption, calculating micropore size distribution from the desorption branch of the N_2 isotherm, (*b*) high-pressure Hg porosimeter, to obtain a macropore size distribution function, and (*c*) measurements of apparent (displacement in Hg) and real (displacement in water) density. Org. matter and water stability were also determined. Soil aggregates from cultivated plots had an internal pore vol. greater than that found in virgin soil aggregates. No positive correlation was found between low org. matter content and water stability, and porous structure. Significant differences of pore structure were found between the soils studied. J. S. C.

Permanent wilting percentage, 15-atmosphere moisture percentage, and hygroscopic coefficient of three soils in Eastern Nebraska. S. A. Miller and A. P. Mazurak (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 260—263).—The lower limit of readily available moisture (that moisture content at which there was no further decrease in % of moisture with time) in this subhumid region was determined down to depths of 36 in. during the growth of sunflower on three soils, the surfaces of which were rainproofed with plastic covers. Values for the lower limit of readily available moisture agree fairly closely with those of permanent wilting point for a sandy loam and a silt loam at all depths and for a silty clay loam to a depth of 12 in.; at lower depths in this last soil permanent wilting point values were rather lower than were those for the lower limit of readily available moisture. The % of moisture retained at 15 atm. tension by the puddled silty clay loam subsoil agreed fairly well with those for the lower limit of readily available moisture. Hygroscopic coeff. were lower than those for the lower limit of readily available moisture for all soils at all depths. A. H. CORNFIELD.

Influence of sample pretreatment on soil moisture retention. D. E. Elrick and C. B. Tanner (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 279—282).—Moisture retention values over the tension range 0·01—15 atm. were determined on samples of eight Wisconsin soils, a comparison being made of samples which had been air-dried and sieved or puddled with those which had been taken as undisrupted cores. With medium-textured soils moisture retention of sieved samples was greater than that of core samples at tensions $<0{\cdot}4$ atm. At tensions $>1{\cdot}0$ atm. water retention by sieved samples was about 10% greater than that by core samples. Puddled samples showed much higher water retention than did core samples over the tension range 0·1—2·0 atm., but not at other tensions. A. H. CORNFIELD.

The cane plant and water. I. Established relationships between soil, plant, and water. Harold R. Shaw (*Sugar, N.Y.*, 1955, **50**, No. 8, 29—31).—The principal factors of soil, plant, and water relationships which influence the design and application of irrigation, especially in reference to overhead sprinkler irrigation, are summarised. Practical application of observations is stressed. The concepts of fundamental importance to good irrigation and max. crop yields are reviewed. E. M. J.

Effect of soil moisture level on yields of, consumptive use of water by, and root development in sugar beet. W. E. Larson and W. B. Johnson (*Proc. Soil. Sci. Soc. Amer.*, 1955, **19**, 275—279).—When sugar beets were allowed to remove 43, 75 and 95% of the available moisture in the root zone prior to irrigation, yields were 23·4, 22·0 and 16·9 tons per acre respectively in an experiment in which plants could not obtain ground water. In an experiment in which the water table was 4—4·5 ft. below the surface yields were similar whether plants were allowed to extract 54% or 66% of the available soil moisture prior to irrigation; yields were slightly less where irrigation was not practised. Consumptive use of water during the growing season was 23·4, 22·5 and 19·0 in. depending on whether 43, 75 or 95% of the available moisture was removed prior to irrigation. Moisture level had no effect on the rate of root extension or the shape of the root. A. H. CORNFIELD.

American tests on soil conditioners. N. H. Pizer (*Agric. Rev.*, 1955, **1**, 61—62).—A short review of recent American experiments. A. G. POLLARD.

Erosion indices for Spanish soils. C. Tamés and M. T. Peral (*Bol. Inst. Invest. agron., Madr.*, 1955, **15**, 61—72).—The erosion index of a soil is a function of the amount and intensity of rainfall, the soil slope, the physico-chemical character of the soil, and the vegetal coverage. Erosion is minimised by a sufficiency of colloidal material in the soil, and increased by high sand content. These points are illustrated by reference to two soil types of widely different character. F. R. PAULSEN.

Wind erosion and dust storms. Anon. (*U.S. Dep. Agric.*, 1955, *Leafl.* 394, 8 pp.).—Facts pertaining to the Great Plains are discussed, with particular reference to the effects of periods of drought and the formation of "dust bowls." Long-term and emergency soil conservation measures are outlined. E. G. BRICKELL.

Chemical and clay mineral properties of a red-yellow podsolic soil derived from muscovite schist. C. I. Rich and S. S. Obenshain (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 334—339).—Profile characteristics of this silt loam soil are described. The soil was nearly devoid of exchangeable Ca and low in other bases. Clay minerals present were kaolinite, dioctahedral vermiculite, and regularly and randomly interstratified illite–vermiculite. *X*-Ray diffraction and differential thermal analysis data of the clays are presented. A. H. CORNFIELD.

Chemical and mineralogical study of clay materials from several gray-brown podsolic soils of Minnesota. A. C. Caldwell, R. S. Farnham and F. L. Hammers (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 351—354).—The chemical and mineralogical characteristics of the $<0{\cdot}5\mu$. clay fraction through the profiles of three grey-brown podsolic soils (silt loams) developed from Peorian loess, non-calcareous glacial till, and calcareous glacial till respectively, are reported. The chemical composition of the clay was similar in all horizons of all the soils. The clay fractions were largely of the montmorillonitic type. A. H. CORNFIELD.

Compression studies of illite suspensions. G. H. Bolt and R. D. Miller (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 285—288).—An apparatus for measuring the swelling pressure of clay suspensions over the range 0·1 to 100 atm. is described. Swelling pressure of Na–illite suspensions in equilibrium with varying concn. of NaCl are reported. The results are discussed in relation to the mechanism of clay flocculation. A. H. CORNFIELD.

Ion exchange in soil-plant root environments. II. Effect of type of clay mineral upon nutrient uptake by plants. D. A. Brown (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 296—300).—Uptake of nutrients by soya-beans grown in sand-clay cultures, containing 15% by wt. of kaolinite–montmorillonite or kaolinite–illite mixtures, each clay mixture ranging from 0 to 100% of kaolinite, was studied. In the kaolinite–montmorillonite series the presence of 2·5% of montmorillonite in the clay mixture resulted in a reduction in the uptake of Ca and Mg as compared with 100% kaolinite. Further increases in the proportion of montmorillonite resulted in increased uptake of Mg, Ca and K. In the kaolinite–illite series uptake of K, Ca and Mg was reduced by inclusion of 2·5—10·0% of illite, but was increased by higher % of illite. pH–titration curves of the two series of mixed clays are also presented. A. H. CORNFIELD.

Rate of phosphate reaction with soil minerals and electron microscope observations on the reaction mechanism. J. A. Kittrick and M. L. Jackson (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 292—295).—The rate of fixation of PO_4''' by four soils was high initially but diminished logarithmically to low values. A latosol (18·2% extractable Fe_2O_3) fixed PO_4''' at a rate ≡100 tons of 20% superphosphate per acre per hr. during the first 3 min. A peat reacted much more slowly. Increasing the PO_4''' concn. of the reacting solution increased the rate of reaction during the first few min. but not thereafter. Over the pH range 2 to 6 max. PO_4''' fixation occurred between pH 2·5 and 4·5. Removal of free Fe_2O_3 from the latosol reduced PO_4''' fixation rate by about 50%. Laboratory prep. of colloidal Fe_2O_3 and $Al(OH)_3$, which had properties similar to materials which cause rapid initial PO_4''' fixation in soils, were prepared. Electron micrographs showed that when these materials were treated with sol. PO_4''' at room temp., at pH 4·3 or 7·0, there was rapid formation of Fe and Al phosphate crystals by the mechanism of solution-pptn. A. H. CORNFIELD.

Effect of soil reaction on the availability of phosphorus for lucerne in some Eastern Ontario soils. A. J. MacLean and R. L. Cook (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 311—314).—Yields of lucerne in pot tests with six soils limed to different pH levels (from about 5·5 to 7·5) increased with soil pH both in the absence and presence of applied superphosphate. All the soils responded to P applications at all pH levels. Total uptake of P by the plant was usually unaffected by changes in pH up to about 6·5, whilst there was a definite increase in P uptake with higher pH. Both native and applied P are most available to lucerne when soil pH is slightly above 7. Liming resulted in increases in acid-sol. (Truog) P in four soils, in adsorbed (Bray) P in two soils, and in $NaHCO_3$-sol. P in five soils. Liming had little effect on adsorbed + acid-sol. (Bray) P in any of the soils. A. H. CORNFIELD.

Radioactive studies with ^{32}P in tropical soils and crops of Puerto Rico. J. A. Bonnet, A. R. Riera and J. Roldan (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 283—284).—Tests with ^{32}P-labelled superphosphate showed that there was much better utilisation of applied P when the fertiliser was broadcast either over the whole canopy area or on a circular segment between 2 and 4 ft. from the base of the tree than

when it was applied in bands or holes in the canopy area or in the area on the slope above the base of the trees. Leguminous trees growing as shade for the coffee trees utilised part of the applied P. A. H. CORNFIELD.

Crop rotations and soil nitrogen. J. L. Haynes and L. E. Thatcher (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 324—327 —Studies over 39 years of some common " corn-belt " cropping systems, showed that, in general, yield levels characteristic of a given rotation were quickly reached and remained at a fairly const. level thereafter. Only where the soil was cropped to continuous maize was there a tendency for yields to continue decreasing with successive years. The total N content of the soil decreased where continuous maize was grown but increased where lucerne or clover were included in the rotation. A. H. CORNFIELD.

Fertiliser studies with radioactive sulphur. II. C. F. Bentley, D. J. Hoff and D. B. Scott (*Canad. J. agric. Sci.*, 1955, **35**, 264—281 ; cf. *Sci. Agric.*, 1951, **31**, 41).—Deficiency of S in the Alberta grey soils can be detected by chemical tests based on determination of the uptake by cereals of legumes of ^{35}S. Considerable uptake was observed on soils of low S contents, usually yielding crops of low S contents. P. S. ARUP.

Trace elements: a gap in nature. D. P. Cuthbertson (*Agric. Rev.*, 1955, **1**, 43—50).—A review of work on the significance of Co, Cu and Mo in animal nutrition. A. G. POLLARD.

Trace element distribution in virgin profiles representing four great soil groups. J. R. Wright, R. Levick and H. J. Atkinson (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 340—344).—The distribution of Zn, Cu, Mn, Mo, Co and Pb by genetically designated horizons in profiles of podsolic, brown podsolic, grey-brown podsolic and brown forest soil groups is presented. Relative to R_2O_3, Zn accumulated in the A_0 and B horizons of podsols and brown podsolic soils and in the A_1 horizons of brown forest profiles. Accumulations of Cu occurred mainly in the A_0 horizons of podsols and brown podsolic soils. When all the soils are considered, Cu was the most easily leached of all the elements studied. The distribution of Co paralleled that of R_2O_3 in gray-brown podsolic and brown forest profiles. The profile distribution of Mn was irregular. The Mo content of all soils was greatest in the B horizon. The A_0 horizons were particularly high in Pb in relation to R_2O_3. A. H. CORNFIELD.

Absorption of radioactive strontium by certain crop plants as influenced by the chemical properties of some Arizona soils. W. J. Flocker (*Dissert. Abstr.*, 1955, **15**, 1168).—The amount of absorption of radio-Sr by various crops from thirteen different soils, with soil Sr-concn. varying from 40 to 320 p.p.m., was determined. No correlation was found between the absorption and the $CaCO_3$ content of calcareous soils, nor with HCl-sol., water-sol., "active," carbonate-, or total Ca soil contents, but a high degree of correlation was found with the exchangeable Ca content of soil. Absorption varied directly with Sr concn. in soil. The concn. of Sr in the edible parts of food crops ranged from 41 to 479 μg. per g. of plant material. J. S. C.

Mobilisation and transport of iron in forested soils. I. Capacities of leaf extracts and leachates to react with iron. W. A. DeLong and M. Schnitzer. **II. Nature of the reaction of leaf extracts and leachates with iron.** M. Schnitzer and W. A. DeLong (*Proc. Soil. Sci. Soc. Amer.*, 1955, **19**, 360—363, 363—368).—I. Over the pH range 3·5–8·5 the Fe saturation capacity [mg. Fe dissolved from freshly pptd. $Fe(OH)_3$ per g. of org. matter present in solution] of aq. poplar leaf extracts or leachates from leaf fall, increased with pH where NaOH was used as neutralising agent ; where $Ca(OH)_2$ was used Fe saturation capacity increased up to pH 6·5 and remained constant with higher pH. Saturation capacity of aq. leaf extracts, leaf fall leachates, or canopy drip varied with species but was always higher at pH 7 than at pH 5. Aq. poplar leaf extracts mobilised Fe and Al from the A_2 and B_2 horizons of podsols and grey-wooded soils.

II. Electrodialysis of poplar leaf extracts enriched with Fe indicated that much of the Fe was present either as $Fe(OH)_3$ or in a form readily convertible to the latter. Much of the Fe was extractable with 8-quinolol in $CHCl_3$. The major part of the capacity of poplar leaf extracts and leachates to react with Fe was isolated by pptn. with 80% aq. EtOH. The principal component of these active fractions had the characteristics of acidic polysaccharides. There was no evidence of chelation between Fe and org. matter either in the original leaf extracts or in the alcohol-pptd. fractions. The primary function of the org. matter is to act as a peptising agent and protecting colloid for $Fe(OH)_3$, although a chemical reaction between Fe and the acidic polysaccharide, R·COOH, to give R·CO·O–$Fe(OH)_2$ may be possible. A. H. CORNFIELD.

Chemical method for the rating of agricultural limestones used as soil amendments. H. E. Gibaly and J. H. Axley (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 301—302).—A chemical method, using ethylenediaminetetra-acetate, for evaluating the liming efficiency of both calcitic and dolomitic limestones of varying particle size and chemical composition is described. Results obtained with a no. of commercial limestones correlated well with both field trials and pH changes of soil suspensions treated with the materials. A. H. CORNFIELD.

Wood sawdust as a source of humus.—Lenglen (*C. R. Acad. Agric. Fr.*, 1955, **41**, 476—481).—An historical review of the subject, covering the past two centuries. J. S. C.

Transformations of sawdust in the course of its decomposition under the influence of *Coprinus ephemerus*. C. B. Davey (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 376—377).—Chemical changes occurring during the composting of sawdust (which had been treated with anhyd. NH_3, neutralised with H_3PO_4 and inoculated with *Coprinus ephemerus*) of hard maple, red oak and jack pine are reported. During composting there was an increase in base exchange capacity and a decrease in holocellulose and α-cellulose in all three materials. There was a decrease in lignin-OMe groups and an increase in lignin in hard maple and red oak composts, but not in jack pine composts. Changes in cold and hot water-sol., 1% NaOH-sol. and alcohol–C_6H_6-sol. fractions are also reported. A. H. CORNFIELD.

Effects of soil extracts and trace elements on growth of various *Streptomycetes*. G. Spicher (*Zbl. Bakt.*, 1955, II, **108**, 577—587).—Evidence is presented which leads to the supposition that the stimulation of the growth of the organisms by the addition to the basic medium of soil extracts is due to the presence therein of trace elements. The validity of the supposition is confirmed by observations on the stimulating effects of traces of Fe (0·05), Mn (0·0005), Zn, Cu and Mo (0·005 mg. per 100 ml. for max. effect), separately or in combination. (25 references.) P. S. ARUP.

Antagonism between soil bacteria and *Colletotrichum atramentarium*, (Berk et Br.).—Taub (*Zbl. Bakt.*, 1955, II, **108**, 602—610).—Antagonism towards the fungus is demonstrated by means of the streak test on nutrient agar for a no. of aërobic spore-forming soil bacteria. The antagonism, in some cases very marked, includes total inhibition of germination of the conidia, but partial inhibition, only, of the sclerotia. Bacterial inhibition on the part of the fungus is observed for one bacterial strain only, which for its part can only inhibit mycelial growth of the fungus. P. S. ARUP.

Effect of calcium cyanamide on pathogenic and non-pathogenic micro-organisms of soil. Hannelore Müller (*Arch. Mikrobiol.*, 1955, **22**, 285—306).—Appreciable changes in soil-pH are effected only by large additions of CaO + $CaCN_2$, and are not responsible for the inhibition of soil-organisms, especially the pathogenic types. On or in suitable nutrient media, moderate additions of $CaCN_2$ stimulate some organisms (e.g., *Penicillia* and bacteria) and inhibit others (mostly pathogenic fungi). Sensitivity, as regards inhibition, to $CaCN_2$ is least (or non-existent) for saprophytic non-pathogenic fungi, and greatest for parasitic pathogenic fungi, *Rhizoctonia solani*, *Phoma betæ*, *Pythium debaryanum*, *Ophiobolus graminis*, and *Thielavia basicola* being particularly sensitive to small additions of $CaCN_2$. The adverse effects of $CaCN_2$ on parasitic fungi, and its utilisation by *Penicillium notatum* are examined in detail. (45 references.) P. S. ARUP.

Commercial fertiliser consumption in the United States, 1953—4. W. Scholl, H. M. Wallace and E. I. Fox (*Farm Chem.*, 1955, **118**, No. 6, 37—50).—A survey of state-by-state data. Single-nutrient fertilisers, mixed fertilisers of various grades and natural organic materials are considered. A. G. POLLARD.

Effects of surface-active agents on caking of stored mixed fertiliser. W. J. Tucker (*J. agric. Food Chem.*, 1955, **3**, 669—672).—Tests are presented of the effectiveness of two surfactants (i) a liquid of an ionic type consisting of an aq. 38% solution of the Na sulphate derivative of 2-ethyl-1-hexanol, and (ii) a non-ionic alkyl phenyl polyethylene glycol ether (95% of active ingredient) in a mixed fertiliser manufactured under controlled conditions. There was no reduction in the caking tendency of the fertiliser in the presence of either of the two surfactants on storage for several months. E. M. J.

Chemical-agricultural problems in the use of anhydrous ammonia as nitrogen fertiliser. D. van Maercke (*Industr. chim. belge*, 1955, **20**, 751—758).—Based on a literature review, a detailed summary is given of research on, and the practical possibilities of, injection of NH_3 gas, or aq. NH_3, into the soil at depths of ~10—15 cm. Data discussed include : intake of ammoniacal N by plants ; methods for and mechanism of fixation of anhyd. NH_3 in soils ; influence of NH_3 on soil acidity, soil constituents and on plants and bacteria ; and results of field tests with anhyd. NH_3 and NH_4 salts, Injection of 36—100 kg. of anhyd. NH_3 per hectare increased yields of grain by 10—17 hl. per hectare on acid soil (pH 5·1) and by 8—21 hl. per hectare on basic soil (pH 7·8). The success of this new method will depend on economic considerations. (27 references.) W. J. BAKER.

Interaction between particle size and water solubility of phosphorus in mixed fertilisers as factors affecting plant availability. K. Lawton and R. L. Cook (*Farm Chem.*, 1955, **118**, No. 4, 44—46).—Effects of granulation of P fertilisers on the distribution of sol. P in soil, after fertiliser treatment, and also the influence of high-analysis formulations of mixed fertilisers are considered. Experimental data quoted suggests that nutritional benefits of granulation are most likely to appear early in the season in crops, e.g., sugar beet, which assimilate considerable amounts of P in early growth. A. G. POLLARD.

Recent progress in the manufacture of phosphate fertilisers. L. E. Andrès (*Chim. et Industr.*, 1955, **73**, 531—540).—The proportion of citrate-sol. phosphates in fertilisers manufactured by the standard processes can be markedly increased by the addition of small amounts of $MgSO_4$ which appear to counteract the opposing effect of fluorides tending to render the phosphates insol. The effect of a large no. of other stabilisers is shown in a series of tables. J.A.C. ABSTR.

Laboratory, greenhouse, and field studies with mixed fertilisers varying in water-soluble phosphorus content and particle size. L. Owens, K. Lawton, L. S. Robertson and C. Apostolakis (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 315—319).—Laboratory tests with mixtures of soil and ^{32}P-labelled PO_4''' fertilisers of varying granule size and having from 2% to 90% of their P in water-sol. form showed that movement of sol. P out of the fertiliser was complete in 24—48 hr. For a given fertiliser, rate of movement of P was independent of soil moisture. The extent of P migration was ∝ the % of fertiliser P which was water-sol. Varying the water-solubility of fertiliser having a similar N : P : K ratio had no effect on dry wt. yields of wheat plants in pot tests. Absorption of fertiliser P by wheat plants from 4—6 mesh granular material was ∝ its water-sol. P content. With powdered fertilisers the water-solubility of the P had little effect on the extent of utilisation of fertiliser P. There was better utilisation of fertiliser P in soil of pH 5·5 than in soils of pH 6·5—7·5. Although there was a very good yield response by sugar beet to applications of P fertiliser in the field, there were no differences in yield between fertilisers containing from 0 to 100% of their P in water-sol. form or between fertilisers of mesh size 6—14 (per in.) or <35. A. H. CORNFIELD.

Formulating liquid fertilisers. R. P. Langguth, J. H. Payne, jun., P. G. Arvan, C. C. Sisler and G. F. Brautigam, jun. (*J. agric. Food Chem.*, 1955, **3**, 656—662).—The methods of formulation which will provide max. nutrient content for solutions having various base ratios are presented. Uusing the common raw materials, total nutrient analysis is rarely more than about 30%. An increased K content results in a lower total nutrient analysis and the max. total nutrient analysis for a given base ratio is generally lower when NH_4NO_3 is substituted for urea as a supplementary source of N. E. M. J.

Cost data for liquid fertiliser plants. W. R. Bone (*J. agric. Food Chem.*, 1955, **3**, 663). E. M. J.

"Brown mud" from the aluminium industry as a soil liming material. C. W. Whittaker, W. H. Armiger, P. P. Chichilo and W. M. Hoffman (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 288—292).—The liming value of "brown mud" (typical analysis SiO_2 23, CaO 47, Fe_2O_3 10, TiO_2 3·5, Al_2O_3 5·5 and Na_2O 3·6% + small amounts of other elements), a waste product from the Bayer process for extracting Al_2O_3 from bauxite was compared with that of $Ca(OH)_2$ and $CaCO_3$. In pot tests with two acid soils brown mud increased yields of sweetclover and uptake of Ca to the same extent as did equiv. amounts of the other liming materials. No toxic effects were noted even when the material was applied at twice the CaO requirement. Uptake of Na was greater, whilst that of K was less, with brown mud than with the other liming materials. The mud increased soil pH to values > those produced by equiv. amounts of the other liming materials. A. H. CORNFIELD.

Correction of trace element deficiencies. F. W. Allerton (*J.R. hort. Soc.*, 1955, **80**, 416—420).—Use of ethylenediaminetetra-acetic acid as a sequestering agent for Fe and Mn is discussed. Significant deficiencies of these elements may be caused by excessive free lime from phosphate fertiliser applications. Prep. containing them, and methods of application are described. E. G. BRICKELL.

Comparative effects of manure and commercial fertiliser in a long-term fertility experiment. W. E. Cordukes, A. J. MacLean and R. F. Bishop (*Canad. J. agric. Sci.*, 1955 **35**, 229—237).—With a crop rotation of mangels, oats, clover and timothy grass, org. manure proved the most efficient for maintaining all the crop yields, and soil quality indices. Inorg. fertiliser by itself, maintained yields of mangels, increased those of oats, but failed to maintain hay yields. Mixed org. and inorg. manuring tended to increase yields of mangels and oats, and to decrease those of hay. Indicator crops of lucerne in greenhouse trials of the respective surface soils from the above experiments, throve better on the soils receiving org. manure than on those receiving inorg. fertiliser alone. P. S. ARUP.

Plant Physiology, Nutrition, Biochemistry

Xeromorphic gradations of cultivated plants. G. L. Farkas and T. Rajhathy (*Planta*, 1955, **45**, 535—548).—Zalensky's observation of a tendency to xerophytic structure in the upper as compared with the lower leaves of plants is confirmed by a study of six mesophytic plants (especially tomato plants). Under reduced water supply, these characteristics, viz. reduction in size of epidermal and stomatal cells, increase in the no. of stomata per unit area, and increased development of palisade and tracheal tissue become more pronounced as a whole, whilst greater differences are observed as between the upper and the lower leaves. Parallel positive relationships are observed for chlorophyll and ascorbic acid contents; negative relationships for water content and succulence are recorded. (21 references.) P. S. ARUP.

Effect of photoperiod on varieties of red clover (*Trifolium pratense*, L.). L. W. Gorman (*N.Z. J. Sci. Tech.*, 1955, **37, A**, 40—54).—The differing reactions of two varieties of red clover, early flowering broad red and the late-flowering Montgomery, to photoperiods ranging from 12 to 16 hr. are recorded. Leaf size, length of petiole and time of commencement of flowering are the most useful characteristics in distinguishing between the two varieties. Differences in size of leaflet and no. of internodes are independent of variations in the photoperiod. N. M. WALLER.

Photoperiodically induced changes in leaf-protein [composition]. H. Metzner (*Planta*, 1955, **45**, 493—534).—A procedure is described by which the leaf-proteins of *Kalanchœ blossfeldiana* can be separated without being denatured by the accompanying tannins; the material is comminuted and washed with $COMe_2$ at ≯ −1 to −2° (keeping the concn. of $COMe_2$ at ≮80%) and finally washed with Et_2O. Paper chromatography of the hydrolytic products of the proteins from the growing points of the leaves reveals 24 NH_2-acids (18 identifiable) which include unusually large proportions of basic and cyclic acids. The changes in NH_2-acid composition due to curtailment of the light period to 9 hr. are complicated during the first seven days by at least two critical turning points in the graphs; initial tendencies are not reflected in the changes observed after 30 days. The findings are discussed from the point of view of "enzymic adaptability." (145 references.) P. S. ARUP.

Nitrogenous constituents in plants. I. Free amino-acids in leaves and leguminous seeds. A. N. Radhakrishnan, C. S. Vaidyanathan and K. V. Giri (*J. Indian Inst. Sci.*, 1955, **37, A**, 178—194).—A qual. survey of the free amino-acid content in the leaves of a no. of species (particularly citrous) and in some leguminous seeds is reported. A detailed study is made of the changes with germination time of the amino-acid distribution in *Phaseolus radiatus*. Germination increases the free amino-acid content to many times its value in the dormant seed. Several unknown ninhydrin-positive compounds including peptides have been detected. (43 references.) A. JOBLING.

External carbohydrates in the growth and respiration of pollen tubes *in vitro*. J. C. O'Kelley (*Amer. J. Bot.* 1955, **42**, 322—327).—Pollen of three plant species germinated readily in 0·32M-sucrose containing a trace of B. Other carbohydrates also favoured the growth of pollen tubes but many showed inhibitory effects. By the use of ^{14}C-labelled sugar the absorption and consumption (in respiration) of externally applied sugars by pollen is demonstrated. Respiration of individual sugars varied considerably in extent. A. G. POLLARD.

Polyfructosans. XLI. Carbohydrate metabolism in *Phleum pratense*. H. H. Schlubach and L. Grassmann (*Liebigs Ann.*, 1955, **594**, 33—41).—The carbohydrate metabolism throughout the vegetation period of *Phleum pratense* has been compared with the principal German pasture grass, *Lolium perenne*, with special reference to the production of albumin, cellulose and lignin at differing cutting periods. The polyfructosan content of *Phleum pratense* behaves in a manner similar to that of inulin in the Jerusalem artichoke, in that a carbohydrate build-up occurs, which serves to supply both flowers and seed, and also functions as a winter food supply. The variations in the conversion of sol. carbohydrate into albumin have also been observed. G. R. WHALLEY.

Flower formation in *Pisum sativum*. II. Effect of sugar nutrition. W. Haupt (*Ber. dtsch. bot. Ges.*, 1955, **68**, 107—120).—Peas were grown without cotyledons under sterile conditions in sugar-containing media (cf. *ibid.*, 1954, **67**, 65). Flowering was favoured by N deficiency in the plants and, to a smaller extent, by deficiency of sugar. Vegetative growth was inhibited more intensely by sugar deficiency than by that of N. Deficiency of both sugar and N induced

flowering at earlier nodes. The N content of plants receiving NO_3'-N was much lower than that of those given yeast-N and only slightly exceeded that of N-deficient plants. The N intake per unit of dry matter was unrelated to the effect of N on flower formation. Accelerated flowering resulting from nutrient deficiency is discussed as an outcome of inhibited vegetative growth. A. G. POLLARD.

Spectral temperature curve of [irradiated] potato parenchyma, and its importance in the study of the effect of light on respiration. G. Rosenstock (*Planta*, 1955, **45**, 591—595).—Increases in temp. in the interior of the tissue, caused by irradiation, are much greater than increases measured in the respiration chamber, and show max. values at $\lambda > 700$ mμ, and then after the spectrum has been broadened to include down to 440 mμ; min. rises occur with the inclusion of decreasing λ after passing the two peaks. Since the above max. coincide with the max. for respiration, further investigation is required in order to determine whether the respiration max. depend on the nature of the irradiation, or merely on secondary heating effects. P. S. ARUP.

Anion antagonisms in yeast as indicators of the mechanism of selenium toxicity. C. W. Bonhorst (*J. agric. Food Chem.*, 1955, **3**, 700—703).—Yeast respiration is strongly inhibited by 10^{-4}M-selenite when glucose or ethanol is used as substrate but not when lactate, pyruvate or acetate is used. Inhibition of yeast respiration by selenite is reduced in the presence of arsenite, arsenate or phosphate. Selenate inhibition is only partially reduced by treatment with arsenate plus phosphate. Selenite or arsenate inhibition is reduced by phosphate, but selenate or arsenite inhibition is not affected. (17 references.) E. M. J.

Methods for determining the viability of various seeds by tetrazolium staining. II. Italian ryegrass and short-rotation ryegrass. E. O. C. Hyde (*N.Z. J. Sci. Tech.*, 1955, **37, A**, 36—39).—Seeds of the two varieties are pre-soaked in water for 16 hr. at 30° and surface-sterilised with Ca hypochlorite. The embryos are then bisected and the seeds steeped in a 1% solution of 2 : 3 : 5-triphenyltetrazolium bromide for 6 hr. at 30°. If all the embryo apart from the coleorhiza and root primordium becomes stained the seeds are regarded as viable. Comparative tests using this and the germination method show that the staining method of viability testing is the more reliable during the after-ripening period. N. M. WALLER.

Assimilation of various sulphur compounds by vegetable tissues cultivated *in vitro*. R. Heller (*C. R. Acad. Sci., Paris*, 1955, **241**, 234—236).—Both healthy and unhealthy carrot tissues, grown *in vitro*, were placed in media containing one of the following: Na_2SO_4, Na_2SO_3, Na_2S, cysteine, glutathione, methionine or taurine. With the exception of the last-named, all were assimilable S sources. Taurine was too toxic to be assimilated. Na_2S, glutathione and methionine were lightly toxic but could be assimilated successfully by healthy tissues. J. S. C.

The rôle of sulphur in the metabolism of living organisms.—Lemoigne (*Rev. Ferment. Industr. aliment.*, 1955, **10**, 103—118).—The biochemistry of S compounds is systematically reviewed. The S compounds found in living matter are classified and their structures defined. The part played by S compounds in general cell metabolism, in animal and plant life and in association with micro-organisms, is examined and the S cycle in nature briefly outlined. J. S. C.

Rôle of molybdenum in soils and plants. E. R. Purvis (*J. agric. Food Chem.*, 1955, **3**, 666—669).—The rôle of molybdenum in soils and plants is reviewed briefly. (46 references.) E. M. J.

Colorimetric determination of phosphorus in plant materials. A. J. Cavell (*J. Sci. Food Agric.*, 1955, **6**, 479—480).—P in plant extracts can be accurately determined colorimetrically as the yellow phospho-vanado-molybdate, using the mixed solution of NH_4 vanadate and NH_4 molybdate. In the presence of HCl colour development to a constant transmittancy takes place in 5 min. if the acidity lies between 0·5N. and 1·5N. E. M. J.

Criteria of essentiality for micro-nutrients, particularly chlorine. E. Malavolta (*Rev. Quím. industr., Rio de Janeiro*, 1955, **24**, 19—20).—The physiological and biochemical criteria for judging essentiality of micro-nutrients are examined and discussed. The essential nature of chlorine for higher plants has been established by Broyer *et al.* (unpublished work), by removal of Cl′, Br′ and I′ from the nutritive solution by Ag^+ pptn.; a severe plant-sickness was induced, which was corrected by restoration of Cl′. H. PRITCHARD.

Chromatography of organic acids in cured tobacco. F. E. Resnik, L. A. Lee and W. A. Powell (*Analyt. Chem.*, 1955, **27**, 928—931).—A method is described for the analysis of some polybasic org. acids in tobacco. One sample is sufficient for the determination of total org. acidity, the qual. identification of these acids by paper chromatography, and their quant. determination by column partition chromatography. The purification of a commercial silica gel is described. In the quant. analysis, Bu^{t}OH–chloroform mixtures are used for eluting the acids from the silica gel column. The eluates are made water-miscible by evaporation of the chloroform, permitting titrations to sharp end-points with dil. aq. NaOH. A fluorescence technique for locating org. acids in paper chromatography has also been developed. The results presented show 96—99% recoveries of acids after silica-gel chromatography. O. M. WHITTON.

Micro-method for continuous and simultaneous determination of CO_2 and O_2 with a modified Warburg apparatus. L. Chapon (*Brasserie*, 1955, **10**, 43—47).—Apparatus and procedures are described for the continuous measurement of O_2 absorbed and CO_2 evolved by the respiration of living tissues. O_2 removal is determined manometrically and CO_2 by measuring conductivity of aq. $Ba(OH)_2$ in which it is absorbed. The sample is placed in a Warburg flask, connected to a manometer and containing a cell of aq. $Ba(OH)_2$ (0·6 c.c.) fitted with platinised Pt electrodes. To prevent inhibition of CO_2 absorption by a film of $BaCO_3$ on the surface, the cell is poised on a tungsten point and oscillated. The calibration of the apparatus and its application to a study of the respiration of dormant barley are described. J. S. C.

Method for studying the influence of aëration and mechanical impedance on seedling roots. W. R. Gill (*Dissert. Abstr.*, 1955, **15**, 1156).—Seedling plants were grown in a transparent cell under an applied pressure opposing that of growth, obtained by the pressure of gas from a high-pressure cylinder exerted on a pneumatically-operated rubber diaphragm. By this means, the relationships of aëration, mechanical impedance and root growth were studied. The rate of growth of maize seedlings varied with O_2 % in the root atm. The applied pressure decreased the growth of seedlings and eventually stopped it (the max. applied pressure at which growth took place was taken as equal to the max. pressure which the root could develop), and the max. root pressure (as thus determined) also varied with O_2 % in the root atm. Increases in salt concn. of the root medium appeared to have little effect on root growth. J. S. C.

Extraction of wax from conifers. O. Härtel and E. Papesch (*Ber. dtsch. bot. Ges.*, 1955, **68**, 133—142).—The approx. wax content of conifer needles is determined by boiling the needles in water and measuring the turbidity of the extract photometrically. Values varied with the age of the needles and with their position on the tree; a seasonal cycle was apparent (high in spring and autumn, low in summer). Prolonged exposure of needles to low concn. of SO_2 increased the turbidity of extracts which thus serves as an index of smoke injury. A. G. POLLARD.

Determination of indol-2-ylacetic acid and its localisation in plant tissues. V. A. Ebert (*Phytopath. Z.*, 1955, **24**, 216—242).—A fluorimetric method, based on the reaction of indol-2-ylacetic acid (**I**) with H_2SO_4 and $CuSO_4$, and capable of detecting 0·2 μg. per ml. of **I** is used for determining (by difference and by comparison with standard solutions) amounts of **I** removed by parts of plants immersed therein. Considerable uptake of **I** is observed for *Cucumis* hypocotyls. Uptake, transport and storage of **I** in seedlings can be studied, under the directions given, by application to the whole plant of the Salkowski (HCl + $FeCl_3$) or the Ehrlich (HCl + dimethylaminobenzaldehyde) reagent; observations on the seedlings (2—10 days old) of seven plants show uptake and storage of **I** to be very considerably greater in the roots (excepting the root-hairs) than in the shoots; active transport of **I** can be observed; storage of **I** in the shoots is limited to regions of growth. Similar results are obtained with indol-2-ylpropionic acid. When applied in conjunction, 1-naphthylacetic acid and **I** appear to act independently of one another by different mechanisms. (61 references.) P. S. ARUP.

Long-term trials on influencing germination and development of *Poa annua* by treatment of the seed with growth-substances. H. Söding and M. Wagner (*Planta*, 1955, **45**, 556—572).—Steeping the seeds in solutions of β-indolylacetic acid, ascorbic acid, or yeast extracts does not affect % germination or the subsequent development of the plants; treatment with β-indolylacetic acid, however, accelerates the germination of immature (but not of mature) seeds, whilst ascorbic acid tends to accelerate germination of the immature seed, provided that it is also applied to the soil, and that light is excluded during steeping and germination; with mature seed, the exclusion of light is not necessary. The presence of active C in the seed bed accelerates germination in all cases. P. S. ARUP.

Detection and chemical separation of the correlated [growth-] inhibitor [of pea plants] and its inhibitor-precursor. E. Libbert (*Planta*, 1955, **45**, 405—425).—The neutral ethereal extracts of pea plants (cf. *ibid.*, 1954, **44**, 286; 1955, **45**, 68) are stable on evaporating their aq. solutions to dryness on the steam-bath, and separable into C_6H_6-insol. and -sol. fractions. The former, obtainable from

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the roots and from etiolated or green shoots, is shown by germination-, root-growth, and bud-tests to be the " correlation inhibitor " itself ; it is unstable in boiling aq. 5% HCl, 1·5% H_2O_2, or M-NaOH. The latter (C_6H_6-sol.) fraction, obtainable from the roots and green leaves and shoots only, further differs from the former in being stable towards HCl, partly stable towards H_2O_2, and inactive in the bud-test ; it is characterised as the inhibitor-precursor. The C_6H_6-sol. and H_2O_2-stable fraction is a weak inhibitor which is active in the germination-, but inactive in the root- and bud-tests ; no physiological importance can be attributed to it. The results of defoliation, decapitation, and de-rooting tests, as regards changes in the contents of the above fractions, are examined and brought into line with the above observations. (22 references.) P. S. ARUP.

Maleimides as auxin antagonists. J. van Overbeck, R. Blondeau and V. Horne (*Amer. J. Bot.*, 1955, **42**, 205—213).—Ten maleimides examined were antagonistic to auxin in plants. A deposit of *N*-1-naphthylmaleimide (0·35 μg. per sq. cm. of leaf) completely defoliated a branch of peach in three days. The effect was counteracted by spraying with naphthylacetic acid. The growth-inhibiting effect of *N*-(2 : 4-dichlorophenyl)maleimide on isolated maize coleoptiles was reversed by indolylacetic acid. The mechanism of the action of maleimides is discussed. Experimental data presented conform with the Muir–Hansch theory of auxin activity.
A. G. POLLARD.

Effects of maleic hydrazide and 2 : 4-D on reducing sugars and sucrose of Red McClure potatoes. M. G. Payne and J. L. Fults (*Amer. Potato J.*, 1955, **32**, 144—149).—Foliage applications of a combined maleic hydrazide (3 lb.)–2 : 4-D (0·5 lb. acid equiv. per acre) spray on July 23 decreased the % of reducing sugars in the tubers at harvest. Application of either material alone on this date had no effect on the % of reducing sugars. Application of both materials, alone or in combination, on Aug. 25 increased the % of reducing sugars. All treatments, with the exception of 2 : 4-D, applied on July 23 increased, whilst none of the treatments applied on Aug. 24 had any effect on the % of sucrose at harvest. The effects of treatments on reducing sugars after 60 days' storage were about the same as those at harvest, whilst their effects on sucrose were not significantly different from untreated controls.
A. H. CORNFIELD.

Effect of preparations of 2 : 4-D and MCPA on growth and formation of conidia of phytopathogenic fungi. F. Wagner (*Arch. Mikrobiol.*, 1955, **22**, 313—323).—The growth of eight fungi in liquid or on solid media is strongly inhibited, but not suppressed by 2 : 4-D or MCPA in concn. of 1·6 g. per l. At lower concn. (e.g., 0·016 g. per l.) mycelial growth may be slightly stimulated, whilst the formation of conidia is very greatly increased. The conidia are slightly stimulated by short, and inhibited by long immersion in aq. 0·1% MCPA. The implications of the above findings as regards effects of the use of chemicals on the dissemination of conidia are considered.
P. S. ARUP.

Inhibition of root growth by streptomycin and reversal of the inhibition by manganese. R. A. Gray (*Amer. J. Bot.*, 1955, **42**, 327—331).—Streptomycin sulphate (**I**) (20 p.p.m.), applied to germinating tomato seeds, inhibited (by 50%) the growth of seedling roots. Root elongation in large tomato plants was also restricted by addition of **I** to the nutrient solution, but not by spray application to the foliage. The action of **I** was partly but not wholly prevented by the presence of Mn. A. G. POLLARD.

Effect of 2 : 4 : 5-trichlorophenoxyacetic acid on cell and nuclear size and endopolyploidy in parenchyma of apricot fruits. M. V. Bradley and J. C. Crane (*Amer. J. Bot.*, 1955, **42**, 273—281).—The increased vol. of peaches sprayed with 2 : 4 : 5-T at the beginning of pit hardening is associated with increased vol. per cell of tissue rather than with a stimulation of cell division. Treated fruits contained larger no. of polyploid cells. A. G. POLLARD.

Use of chemical thinning sprays on apple trees in New Zealand. II. Further experiments with dinitro compounds and synthetic growth substances. R. M. Davison (*N.Z. J. Sci. Tech.*, 1955, **37, A**, 1—7).—An account of experiments with α-naphthylacetic acid, Na dinitro-*o*-cresylate and other chemical thinning agents. Effects on foliage, russeting of apples, blossom formation, fruit size and yields, and maturity differences between sprayed and unsprayed fruit are recorded. Both chemicals are regarded as promising thinning agents, particularly for Sturmer apples.
N. M. WALLER.

Apple blossom thinning with sodium naphthylacetate. D. Barak (*Hassadeh*, 1954, **34**, 488).—Effects of spraying with Na naphthylacetate (10—20 p.p.m.) on a no. of apple varieties were examined. Poor cropping varieties should not be thinned by this means. The spray should be applied within a fortnight of the beginning of petal-fall and may be mixed with customary fungicides or insecticides. It should be applied chiefly to the upper and outer portions of the tree. HORT. ABSTR. (A. G. P.).

Types of flowers in " brinjal " (*Solanum melongena*), based on style length, and their fruit-set under natural conditions and in response to 2 : 4-D as a plant-growth regulator. S. Krishnamurthi and D. Subramanian (*Indian J. Hort.*, 1954, **11**, 63—67).—Four types of flowers are distinguished according to their length of style. Of 1450 flowers examined 93% of those setting fruit normally were long-styled. Treatment with 2 : 4-D increased fruit-set up to 100% in long-styled flowers and to smaller extents in those having intermediate style lengths but had no effect on true short-styled flowers. A. G. POLLARD.

Stimulation of yield in *Hevea braziliensis*. I. Pre-war experiments with vegetable oils. E. D. C. Baptist. **II. Effect of synthetic growth substances on yield and on bark renewal.** E. D. C. Baptist and P. de Jonge. **III. Further observations on the effects of yield stimulants.** P. de Jonge (*J. Rubb. Res. Inst. Malaya*, 1955, **14**, 355—361, 362—382, 383—406).—I. A light scraping of the bark 12 in. below the tapping cut results in large increases in yield which are further enhanced by the application of palm oil to the scraped bark.

II. In recent studies most marked responses are recorded with salts and esters of Cl-substituted phenoxyacetic acids similarly applied. Injections of $CuSO_4$ (5 g. of finely powdered salt introduced into each of two small holes 2·5—3 in. deep) into the trees gave comparable responses in yield.

III. The response to yield stimulants is influenced by the condition of the bark, the type of planting material, the tapping system, the composition and method of application of the mixture. Increase in the area of rubber extraction as a result of treatment is thought to account for increase in yield and for the low incidence of brown bast ; increase in bark thickness is caused by induced meristematic activity in the cortical and phloem regions ; the rate and duration of latex flow are increased after treatment. (15 references.)
E. M. J.

Crops and Cropping

Effect of crop rotations and soil treatments on soil productivity. J. A. Hobbs (*Proc. Soil Sci. Soc. Amer.*, 1955, **19**, 320—324).—Yields of crops over 42 years using a 16-year rotation (4 years lucerne, 12 years grain), a maize–legume–wheat rotation, or continuous cropping to wheat, maize, or lucerne, all rotations being subjected to differential fertiliser treatment, on an alluvial loess soil are reported. Higher yields were obtained in the 16-year rotations than in the other systems. Manure and fertilisers increased yields in general, but yields of maize were reduced by P applications. Yields of unfertilised wheat and maize were maintained with time only in the 16-year rotation. Yields of wheat treated with NPK increased, whilst those of lucerne decreased with time in all systems irrespective of manurial treatment. A. H. CORNFIELD.

Nitrogen fertilisation of winter wheat.—Gros (*C. R. Acad. Agric. Fr.*, 1955, **41**, 421—422).—The advantages and disadvantages of the late application of N fertiliser, according to Coïc's method, in the different regions of France are discussed. In trials, most farmers favour the second application at the growth-of-stalk stage ; application at ear-formation is completely excluded in the South ; in Northern France it is unnecessary, but both of these later applications of N may be of value in Central, Eastern and Western France.
E. M. J.

Growth of pasture species. II. Perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and paspalum (*Paspalum dilatatum*). K. J. Mitchell (*N.Z. J. Sci. Tech.*, 1955, **37, A**, 8—26 ; cf. *ibid.*, 1954, **36**, 193).—The effects of temp., defoliation and shading on the pattern of vegetative development of young plants of perennial ryegrass, cocksfoot and paspalum are determined. Differences in growth habit are discussed in relation to suitability of a species for different types of sward. Under all conditions, except shading at 83°F., the increase in tiller no. is faster in perennial ryegrass than in cocksfoot or paspalum. N. M. WALLER.

Fertiliser, cultivation and grassing trials with pampas grass (*Cortaderia selloana*). W. A. Jacques (*N.Z. J. Sci. Tech.*, 1955, **37, A**, 55—61).—The response to fertiliser application and the effect of cultivation and ground flora on yield of pampas grass are determined. Clean cultivation has an early beneficial effect on yield, and a significant response is observed to the application of superphosphate alone or with sulphates of ammonia and potash.
N. M. WALLER.

Plant proteins. IV. Amino-acids present in alcoholic extracts of grass and legume hays and fenugreek seeds. J. Kolouŝek and C. B. Coulson (*J. Sci. Food Agric.*, 1955, **6**, 455—461).—The amino-acid content of alcoholic extracts of lucerne hay cut at five different stages of growth, and of mature grass and legume hays was deter-

mined by a paper chromatographic method. The contents of most of the amino-acids decline quantitatively with age, this presumably being caused by fall-off in metabolism. There was a distinct difference between the unmanured lucerne, cut in various stages of growth, and mature lucerne hay and the other mature hays obtained from fertiliser-treated plots in the contents of aspartic acid–asparagine and of glycine, serine, alanine and proline. The alcoholic extract of fenugreek seeds contained few free amino-acids; others, observed after hydrolysis, were presumably present originally in peptide form. (23 references.) E. M. J.

Effect of moisture and phosphate variables on lucerne hay production on the Yuma Mesa. C. O. Stanberry, C. D. Converse, H. R. Haise and O. J. Kelley (*Proc. Soil Sci. Soc. Amer.*, 1955, **19,** 303—310).—The effects of different moisture levels, through irrigation, and of varying rates and/or frequencies of superphosphate application (100—1300 lb. P_2O_5 per acre over four years) on yields of lucerne hay on loamy fine sand over four years are reported. Yields increased with moisture level and P applications, although nearly optimum yields were obtained with 200 lb. of P_2O_5 at seeding time followed by annual applications of 100 lb. of P_2O_5. Applied P did not penetrate more than 18 in. Water used per ton of hay produced varied from 7·1 to 16·5 in., the greatest efficiency of water use occurring with the heavy irrigation and P treatments.
A. H. CORNFIELD.

Boron requirements of stone fruit trees. C. G. Woodbridge (*Canad. J. agric. Sci.*, 1955, **35,** 282—286).—Deficiency of B in sand cultures (at 0—0·1 p.p.m. of added B in the nutrient solution) caused early die-back of the branches of peach, apricot, prune or cherry trees. The symptoms caused by excess of B (at 5—10 p.p.m.) varied considerably. Relationships are observed between the B contents of the leaves and twigs, and the nature and severity of the symptoms. P. S. ARUP.

Temperatures in relation to deciduous fruit production. Dillon S. Brown (*Food Technol.*, 1955, **9,** 409—411).—A brief review dealing especially with apricots and cling peaches. Temp. during the first growth period, by accounting for the greatest variable in determining the seasonal rate of fruit development, serve satisfactorily as the basis of estimating harvest time. E. M. J.

Varieties of olive cultivated in Spain. J. M. Ontega Nietr (*Inst. nac. Invest. agron., Madrid*, 1955, 75 pp.).—An illustrated description of the botanical characteristics of plants, leaves and fruit and the agronomy of 24 varieties of olive grown in different areas in Spain. H. S. R.

Two new cane varieties released. Anon. (*Sugar, N.Y.*, 1955, **50,** No. 8, 43).—The varieties (C.P. 48-103 and C.P. 47-193) were released under the joint sanction of the U.S. Dep. of Agric., the Louisiana St. Exp. Sta., and the Amer. Sugar Cane League. The characteristics of each are described. C.P. 48-103 should be grown only on fertile, well drained light soils; C.P. 47-193 is said to do relatively well on heavy soils. E. M. J.

Qualitative differences in the alkaloid fraction of cured tobaccos. R. N. Jeffrey and T. C. Tso (*J. agric. Food Chem.*, 1955, **3,** 680—682).—A detailed study of nicotine-type alkaloids is presented, these being characteristic of all tobaccos; however the chemical composition of the leaf may be influenced by variety, climate, soil, etc. Of the two principal alkaloids nicotine and nornicotine, when nornicotine occurred in greater proportion the sample contained more myosmine than that contained in samples with the greater proportion of nicotine. In the samples tested no evidence was found of any consistent difference in the proportion of the individual nicotine-type alkaloids related to tobacco type. E. M. J.

Interaction of sodium and potassium on growth and mineral content of flue-cured tobacco. E. T. McEvoy (*Canad. J. agric. Sci.*, 1955, **35,** 294—299).—Symptoms due to K-deficiency are appreciably mitigated in the young plants by additions of Na to the nutrient solutions, but are manifested in later growth. With adequate supplies of K, additions of Na reduce yields of fresh plant material. Yields are significantly affected by variations in supplies of K, but not of Na. Accumulations of K or Na depend on the amounts supplied; K depresses Na uptake, whilst K + Na depress the uptake of Ca, Mg and P. P. S. ARUP.

Agronomic and economic advantages of the cultivation of groundnuts in high density. M. Ollagnier and D. Gros (*Oléagineux*, 1955, **10,** 547—555).—Field trials in French Africa were carried out on an extensive scale to determine the effect of sowing density on yield of groundnuts. The results are reported in detail and it is concluded that, for erect, late varieties, sowing density can be increased with advantage from 80,000 to 110,000 grains/hectare; for sloping varieties (common in central and north Senegal), the density of 110,000 grains/hectare should be retained; and for the erect, early varieties, the figure can be even further increased to 160,000—200,000. (16 references.) J. S. C.

Effect of certain site and soil factors on the establishment of Douglas-fir on the Tillamook Burn. G. L. Lowry and C. T. Youngberg (*Proc. Soil Sci. Soc. Amer.*, 1955, **19,** 378—380).—The survival of Douglas-fir seedlings as related to various physical and chemical characteristics of the soil are reported. Of the factors affecting survival the most significant was the no. of days in which the soil moisture content was below the 15 atm. tension level. High mortality occurred in light-textured soils where moisture level was below this value for about 30 days. Seedling survival was poorer on southerly than on northerly aspects, and was usually poor when slopes were >50%. A. H. CORNFIELD.

Fatty acids of the root of *Taraxacum kok-saghyz*. T. Horche, E. Rodriguez, J. Jimenez, J. L. Linaza and W. De Rafols (*An. Inst. Invest. agron., Madr.*, 1955, **4,** 153—159).—The fatty acid fraction of the root of *Taraxacum kok-saghyz* contains palmitic, stearic, oleic, linolic and linolenic acids. The unsaponifiable fraction contains the triterpene alcohol taraxerol in the uncombined state. Details are given of the chemical prep. and examination of these fractions. F. R. PAULSEN.

Industrial evaluation of *Taraxacum kok-saghyz*. E. Rodriguez, T. Horche, J. Jimenez Herrera, J. L. Linaza and W. De Rafols (*An. Inst. Invest. agron., Madr.*, 1955, **4,** 133—144).—Samples of *Taraxacum kok-saghyz* were extracted successively with light petroleum, ether, $CHCl_3$, EtOH (pure), 70% EtOH, cold water, boiling water, 1% HCl and 5% NaOH, and the various fractions are discussed. Of greater interest are fractions containing triterpene alcohols, phytosterols, fats and resins. Phenols, glucosides, bases, flavones and proteins are also present in varying amounts. Carbohydrates include pentosans, mucilages and hemicelluloses.
F. R. PAULSEN.

Determination of copper in plant materials by means of zinc dibenzyldithiocarbamate. S. Andrus (*Analyst*, 1955, **80,** 514—516).—A method is described for the rapid absorptiometric determination of Cu (0—15 p.p.m.) in plant materials. The sample is wet ashed with H_2SO_4, HNO_3 and $HClO_4$, the diluted digest is extracted with a solution of Zn dibenzyldithiocarbamate in CCl_4, and the optical density of the yellow extract is measured at 440 mμ., the Cu content being ascertained from a calibration graph prepared by extraction of standard $CuSO_4$ solutions. Recovery is good and there is no interference from other metals commonly occurring in plant material. A. O. JONES.

Pest Control

Recent trends in insecticide research. L. K. Cutkomp (*Trans. Amer. Ass. Cereal Chem.*, 1955, **13,** 83—101).—A review with 154 references. P. S. ARUP.

Chemical names for active ingredients of fungicides. S, E. A. McCallan, L. P. Miller and M. A. Magill (*Phytopathology*, 1955, **45,** 295—302).—About 200 names of proprietary fungicides with their active ingredients are listed. A separate list of the chemical names of the constituents is given alphabetically. A. G. POLLARD.

Common names of insects. F. J. Gay (*Sci. ind. Res. Org., Melbourne*, 1955, *Bull.* No. 275, 32 pp.).—Two lists give (*a*) the scientific names of insects and related pests in alphabetical order followed by the common name and (*b*) the common names in alphabetical order followed by the scientific names. The common names are those used in Australia. J. S. C.

West African insecticides. F. R. Irvine (*Colon. Plant Anim. Prod.*, 1955, **5,** 34—38).—The active principles of natural insecticides used in West Africa and their effects are surveyed briefly.
S. C. JOLLY.

Detection of dichlorodiphenyltrichloroethane (DDT). L. Garbe and G. Krippner (*Chem. Tech., Berlin*, 1955, **7,** 424).—A rapid test for DDT is described; a small sample is suspended in acetone, alcoholic KOH is added, the solvent is evaporated off, and a drop of a solution of 0·25 g. of K dichromate in 100 c.c. of conc. H_2SO_4 is added. The presence of DDT is shown by a carmine-red coloration. Some modifications to the procedure are described, particularly for cases where the DDT is present in μg. quantities, or where other liquid products are present; 1 μg. of DDT can be detected. The test is more sensitive than that based on the pale carmine coloration produced by DDT in solvents such as ether or acetone with acid dichromate. H. L. WHITEHEAD.

Fungicidal action of sulphur on powdery mildew of vines. A. F. Wilhelm (*Weinberg u. Keller*, 1954, **1,** 124—129).—The action of S on the mildew is not due to formation of SO_2 but to vaporisation of

elementary S at temp. about 20°. The S mol. under these conditions penetrates into the protoplasm of the fungus where it is reduced to H_2S. HORT. ABSTR. (A. G. P.).

Effects of chlorinated hydrocarbon insecticides on quail and pheasants. J. B. De Witt (*J. agric. Food Chem.*, 1955, **3**, 672—676).—Feeding of diets containing 0·02% of DDT to breeding quail resulted in significant decreases in hatchability of eggs and in viability of chicks. Similar results were obtained by feeding 0·001% of Dieldrin, but effects on reproduction of short-term feeding of Aldrin and Endrin could not be determined. Aldrin, Dieldrin and Endrin were lethal to both male and female quail when fed at levels of 0·0005% in the diets. Female pheasants appeared to be more resistant than were males to the effects of these compounds. (13 references.) E. M. J.

Excretion of heptachlor epoxide in the milk of dairy cows fed heptachlor-sprayed forage and technical heptachlor. R. E. Ely, L. A. Moore, P. E. Hubanks, R. H. Carter and F. W. Poos (*J. Dairy Sci.*, 1955, **38**, 669—672).—Heptachlor (**I**) and heptachlor epoxide (**II**) were not excreted in the milk of cows fed hay made from forage harvested seven days after spraying with 3·8—8·0 oz. of **I** per acre. **II** was detected in the milk of cows fed 1·3 mg. of **I** per kg. of body wt. Milk from animals fed ≯3·78 mg. of **I** per kg. of body wt., contained **II** (up to 5·7 p.p.m.). From 0·3 to 2·8% of **I** fed was excreted in the milk as **II**. S. C. JOLLY.

Synthesis of simple quinone derivatives with fungicidal, bacteriostatic or cytostatic properties. S. Petersen, W. Gauss and E. Urbschat (*Angew. Chem.*, 1955, **67**, 217—231).—A detailed discussion is given of the prep. and properties of the simple quinone deriv. possessing fungicidal, bacteriostatic or cytostatic properties, and their use in combating moulds in seed and plants, diseases in growing plants and certain animal ailments. The effects of the structure and substituents on the properties are described and the specific action of a very large no. of compounds is given. Particularly important deriv. discussed are: quinoneoxime benzoylhydrazone (made from benzhydrazide and nitrosophenol) which is useful for seed treatment to impart protection against mould, etc., and also protection to the growing plants against disease; quinone 1-guanylhydrazone 4-thiosemicarbazone which has good bacteriostatic properties especially against streptococci; and 2 : 5-bisethyleneiminoquinone which shows remarkable cytostatic effects in animal experiments, especially against Yoshida sarcoma in rats. (44 references.) J.A.C. ABSTR.

Effect of droplet size and the rate of application in controlling insects and diseases of row crops. John Ralph King (*Dissert. Abstr.*, 1955, **15**, 1156—1157).—Cu oxychloride-sulphate was applied at a constant rate per acre to bean and onion foliage by a mist-spraying machine, varying the droplet size (110 and 175μ. mass median diameter) and rate of application (10 and 20 gal./acre). Leaf print evaluations showed that the 20 gal. rate and larger droplets gave the best coverage. Insect and disease control experiments were carried out, comparing the mist sprayer with a low-vol. sprayer and a duster. The mist spray gave the best control of onion blast due to *Botrytis allii*, Munn. and the low-vol. sprayer gave best control of onion thrips, *Thrips tabaci*, Lind. All spray treatments were equally effective in suppressing Mexican bean beetle larvæ, *Epilachna verivestis*, Muls. J. S. C.

Colorimetric analysis of *p*-chlorobenzyl *p*-chlorophenyl sulphide (Chlorbenside) residues in plant and animal tissue. D. J. Higgons and D. W. Kilbey (*J. Sci. Food Agric.*, 1955, **6**, 441—448).—The method described depends on the pre-oxidation of spray deposits of Chlorbenside to its corresponding sulphone with H_2O_2 in glacial acetic acid, and nitration of the sulphone to its corresponding trinitro-derivative, which in benzene develops a purple colour with Na methylate. The colour, which is not stable, reaches a reproducible peak 1—2 min. after development. The method is sensitive to 0·05 mg. and is specific in the presence of normal impurities and other spray materials except DDT. E. M. J.

Pre-assay purification of tissue extracts by wax column. W. R. Erwin, D. Schiller and W. M. Hoskins (*J. agric. Food Chem.*, 1955, **3**, 676—679).—A method is described for removing interfering substances which have been extracted from plant or animal tissues in the determination of residues of org. insecticides. A column consisting of finely ground alumina coated with a 2 : 1 mixture of white petrolatum and paraffin wax (m.p. 160—165°F.) is used. The column is eluted with aq. acetonitrile (60%), but a separation of the toxicants may be effected by the successive use of 40, 60 and 75% solutions. Recovery of toxicant is satisfactory (e.g. 90—100% for DDT, toxaphene, etc. from spinach). E. M. J.

"Foot disease" in cereals from Aragon. Agustin Alfaro (*Bol. Inst. Invest. agron., Madr.*, 1955, **15**, 159—185).—"Foot-disease" or "black-foot" which affects the base of cereal culms, may be due to several infecting fungi (especially *Ophiobolus graminis*, Sacc., *Leptosphæria herpotrichoides*, de Not, *Wojnowicia graminis*, McAlp., and *Helminthosporium sativum*). Mycelial characteristics and their effects are described and illustrated, and methods of combating the condition suggested. F. R. PAULSEN.

Seed treatment of forage legumes and grasses with three antibiotics. R. S. Fulkerson and W. E. Tossell (*Canad. J. agric. Sci.*, 1955, **35**, 259—263).—Successful results obtained with aureomycin, penicillin and Terramycin in greenhouse experiments with two of the five plants under trial could not be repeated in field experiments. Treatment of grass seeds gave negative results throughout. P. S. ARUP.

Distribution and pathogenicity of fungi associated with crown and root rotting of lucerne in Manitoba. W. C. McDonald (*Canad. J. agric. Sci.*, 1955, **35**, 309—321).—A survey, based on laboratory and field observations, is given in which the prevalent fungi are classified according to their occurrence with respect to soil-types, season, age of plants attacked, and symptoms produced. P. S. ARUP.

Immunity to virus-X in potato: studies of clonal lines. R. G. Timian, W. J. Hooker and C. E. Peterson (*Phytopathology*, 1955, **45**, 313—319).—Means of identifying immunity to virus-X in potato plants are examined. *Gomphrena globosa* served as an indicator plant. A. G. POLLARD.

Treatment of potatoes infected with virus diseases. G. Morel and C. Martin (*C. R. Acad. Agric. Fr.*, 1955, **41**, 472—474).—The meristem of a virus-infected potato is generally free from the infection. Meristem fragments from an infected potato plant were cultivated *in vitro* in a nutrient solution, grafted on a mature tomato plant and planted. Healthy plants were obtained by this means with several varieties of potato. J. S. C.

Controlling the apple sawfly. E. P. Gorjačeva and M. B. Beljahova (*Sad i Ogorod*, 1954, No. 5, 78—79).—In the Leningrad area best results in controlling the sawfly were obtained by applying BHC dust (150 g. per tree) to the soil prior to blossoming before the insects appeared and again during the June "drop" when the caterpillars enter the soil. HORT. ABSTR. (A. G. P.).

Chemotherapy of the peach rosette virus with antibiotics. G. KenKnight (*Phytopathology*, 1955, **45**, 348).—Partial control of the virus on seedling peach trees was obtained by dipping the trees in solutions of various cycline-deriv. A. G. POLLARD.

Use of diphenyl wraps against decay in N.Z. lemons. W. A. Fletcher (*N.Z. J. Agric.*, 1954, **88**, 115—120).—Fruit previously dipped in 2 : 4 : 5-T to control stem-end rots was afforded additional protection from moulds etc. during prolonged storage, by wrapping in papers impregnated with diphenyl. A. G. POLLARD.

Use of zinc ethylenebisdithiocarbamate as a dust. R. Ciferri (*Not. Mal. Piante*, 1954, **27**, 7—14).—Dithane Z-78 effectively controlled downy mildew of vines when applied during flowering and four times subsequently at 10–14-day intervals. Dithane + S was equally effective and more economical. Dusting gladiolus with Dithane gave protection against *Fusarium oxysporum gladioli* and *Penicillium gladioli*. When applied to geranium cuttings Dithane gave better control of basal rot (*Pythium*) than did ZnO, and stimulated rooting. HORT. ABSTR. (A. G. P.).

Tests with organic fungicides against downy mildew of vines. D. Boubals and A. Vergress (*Progr. agric. vitic.*, 1954, **141**, 330—335; **142**, 6—12).—The residual action of *N*-trichloromethylthiotetrahydrophthalimide (**I**) and Zn ethylene-2-dithiocarbamate (**II**) was brief; that of **II** but not of **I** was proportional to the concn. applied. When either is used a final treatment with Bordeaux mixture is needed. HORT. ABSTR. (A. G. P.).

Strawberry vein-banding virus. N. W. Frazier (*Phytopathology*, 1955, **45**, 307—312).—The process and timing of the transmission of the virus mechanically, by dodder, by grafting and by aphids, notably, *Capitophorus fragæfolii*, are examined. A. G. POLLARD.

Control of soil sickness in orchards. H. Lundstedt (*Fruktodlaren*, 1954, No. 2, 52—55).—Comparison is made of four treatments for sick soil. Beneficial effects of the treatments (increased stem diameter) were in the descending order, (*a*) replacement of soil round the tree (1·5 sq. m., 30 cm. deep) by similar fresh soil, (*b*) use of formaldehyde, (*c*) fumigation with DD, (*d*) application of a tar prep. HORT. ABSTR. (A. G. P.).

Screening tests with fungicides for control of French-bean rust [*Uromyces appendiculatus*, (Pers.) Unger]. H. Jacks and R. M. Brien (*N.Z. J. Sci. Tech.*, 1955, **37, A**, 62—67).—Of 37 fungicides tested in the glasshouse for control of French-bean rust the effective compounds were: lime-sulphur with colloidal S, colloidal S, fine wettable S, zineb, ferbam, Maneb, thiram, ziram, Captan, Dichlone, chloranil and nitrobenzene. N. M. WALLER.

Lettuce root rot studies. C. I. Hannon (*Dissert. Abstr.*, 1955, **15**, 1159).—Studies of a root rot disease, recently prevalent in New York State in lettuce crops grown on muck and which produces chloronemia, wilting and stunting of the plants, are reported. A form of *Pythium vexans*, de Bary, was isolated in pure culture only in the earliest stages of the disease and the isolates caused some stunting and death in a no. of inoculated plants but not the full symptoms of the disease found in the field. Later, *Fusarium* and *Cephalosporium* spp. were isolated from rotted roots but the isolates did not appear to cause infection in greenhouse plants. The disease appears to be favoured by low temp. and high soil moisture. Lettuce planted in late June and July is less susceptible to it than lettuce planted early in spring. Several varieties of cos and butterhead lettuce appear to be highly resistant or field-immune to the disease. J. S. C.

Nature of *Fusarium* resistance in tomato. S. S. Gothoskar, R. P. Scheffer, M. A. Stahmann and J. C. Walker (*Phytopathology*, 1955, **45**, 303—307).—Experimental evidence recorded indicates that resistance cannot be ascribed to the existence of a detoxicating mechanism occurring only in resistant plants but that it is associated with metabolic processes and probably results from a labile substance for the production of which energy from respiratory processes is utilised. A. G. Pollard.

Organic mercury dips for the control of nematodes in roots of living plants. J. Feldmesser and W. A. Feder (*Phytopathology*, 1955, **45**, 347).—Of 15 fungicides examined, Captan (2 lb. of 50% wettable prep. per 100 gal.) and wettable S (6 lb. per 100 gal.) were the most effective, Captan giving generally the better results. A. G. Pollard.

Effect of zineb (Dithane Z-78) on the survival of cotton seedlings. D. W. Young and G. A. Brandes (*Phytopathology*, 1955, **45**, 350).—Seedling losses were diminished and subsequent yields of lint were increased by application of suspensions of zineb (3·25 lb. per acre) in the seed furrow. From diseased seedlings *Rhizoctonia*, *Fusarium* and *Pythium*, with smaller no. of *Rhizopus*, *Verticillium* and *Alternaria*, were isolated. A. G. Pollard.

South American leaf blight. R. N. Hilton. **Appendix: Memorandum South American leaf disease of rubber.** R. A. Altson (*J. Rubb. Res. Inst., Malaya*, 1955, **14**, 287—337, 338—354).—The literature is reviewed including the occurrence of the disease in South America, its threat to the Far East, and the methods available for control. The life history of the causal organism, *Dothidella ulei*, is described, and legislation against the disease is discussed. (105 references.)

On account of the increasing volume and speed of air traffic, Malaya has been brought within critical range of infection by *Fusicladium* spores (conidial stage of *D. ulei*) which survive desiccation up to 15 hr. This potential threat to the Malayan rubber industry, the means for defence against introduction of the disease, and measures which might be adopted to deal with it, are discussed. E. M. J.

[A] **Variable effects of nematocides on parasitic nematode populations in row-fumigated tobacco plots.** C. J. Nusbaum. [B] **Comparison of Dowfume W-85, and D-D mixture applied as liquids and impregnated on vermiculite for nematode control.** C. J. Nusbaum and J. N. Sasser (*Phytopathology*, 1955, **45**, 349, 349—350).—[A] Applications, between rows of tobacco plants by gravity-flow apparatus, of Dowfume W-85 (1 : 2-dibromomethane) (**I**) 0·65 ml., D-D 3·26 ml. or PN20 (20% of **I** in Phillips hydrocarbon mixture) 2·44 ml. per linear ft. of row, gave satisfactory control of root-knot nematodes (*Meloidogyne* spp.). Both prep. of **I** markedly reduced the no. of *Tylenchorhynchus claytoni* and practically eliminated *Helicotylenchus* spp. and *Xiphinema americanum*. D-D was less effective on these species.

[B] The above fumigants in the form of impregnated vermiculite gave slightly better results than when applied in the liquid form between rows at 8 in. depth two weeks before sowing. A. G. Pollard.

Present state of research on inactivation of pathogenic viruses, especially tobacco mosaic virus. W. Bartels (*Phytopath. Z.*, 1955, **24**, 117—178).—A review and discussion of the literature, including tabulated results for the effects on virus activity of chemicals, bacteria and moulds, and of plant juices. (101 references.) P. S. Arup.

Marine borer studies: evaluation of toxicants. P. C. Trussell, C. O. Fulton, C. J. Cameron and B. A. Greer (*Canad. J. Zool.*, 1955, **33**, 327—338).—Of 115 compounds tested against *Bankia setacea*, the most toxic was Na_3AsO_3, which gave a complete kill at 25 p.p.m. (as As_2O_3) after 18 hr. exposure. (16 references.) E. G. Brickell.

The rôle of nucleic acids in virus multiplication. R. Jeener (*Rev. Ferment. Industr. aliment.*, 1955, **10**, 122—124).—The evidence that the genetic and reproductive functions of viruses and bacteriophages are limited to the deoxyribose nucleic acid structure present in the organism is reviewed. J. S. C.

Development of sampling techniques for forest insect defoliators, with particular reference to the spruce budworm. R. F. Morris (*Canad. J. Zool.*, 1955, **33**, 225—294).—Work carried out on the Green River Watershed in New Brunswick is reported and covers the objects, timing, and methods and procedures of sampling, together with selection, correlation and transformation of data. (36 references.) E. G. Brickell.

***Botrytis* disease of gladiolus due to *Botrytis gladiolorum*, Timmermans.** C. Bruhn (*Phytopath. Z.*, 1955, **24**, 179—194).—An account of recent occurrences of the disease in Germany, covering symptoms, predisposing factors, and precautionary measures. (35 references.) P. S. Arup.

New principle of weed control. R. L. Wain (*Agric. Rev.*, 1955, **1**, 25—31).—A short summary of recent development in the use of some synthetic herbicides. A. G. Pollard.

Weed control in wheat, barley, potato and lucerne crops. L. J. Matthews (*N.Z. J. Agric.*, 1954, **88**, 103—104).—The Bu ester of MCP (**I**) and the polyethylene glycol ester of 2 : 4-D (**II**) were more toxic than were other formulations tested to weeds usually difficult to kill (bindweed, dock, willow-weed). Both herbicides caused a diminution in length of wheat straw but did not affect grain yields. The action of **I** was more selective than that of **II** and hence **I** is the more suitable for the more susceptible cereals (maize, spring oats). On potato soils pre-emergence applications of TCA (20—40 lb. per acre) destroyed rhizomatous grasses. Post-emergence applications of 2 : 4-D prep. decreased potato yields but the Na salt of MCP had no ill-effects. Grasses in lucerne crops were controlled by powdered IPC (3—6 lb. per acre) as a pre- or post-emergence application and broad-leaved weeds were eliminated by DNBP applied at the 2—3-leaf stage. IPC did not give effective control of rhizomatous or stoloniferous grasses for which dormant season treatments with TCA gave much better results. A. G. Pollard.

Synthesis of some β-aryloxyethanols and aryloxyacetones, and their physiological activity in plants. K. S. Bokarev and N. N. Mel'nikov (*Zh. obshch. Khim.*, 1954, **24**, 2014—2023).—The compounds $OH{\cdot}CH_2{\cdot}CH_2{\cdot}OR$, most of which are new, have been synthesised (45—80% yields) from NaOR and $CH_2Cl{\cdot}CH_2{\cdot}OH$ [R = 4-C_6H_4Br (**I**), 2 : 4-$C_6H_3Cl_2$ (**II**), 2 : 4 : 5-$C_6H_2Cl_3$ (**III**), 2- and 3-$OMe{\cdot}C_6H_4$, 2-$OEt{\cdot}C_6H_4$, 4 : 2-$OAlk{\cdot}C_6H_3Cl$ (Alk = Pr, Bu, C_5H_{11}), 4 : 2-$OBu{\cdot}C_6H_3Br$, 2 : 4 : 5-$OAlk{\cdot}C_6H_3X_2$ (Alk = Me, Et, Pr, Bu, C_5H_{11}; X = Cl, Br)]; $OH{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}C_{10}H_7$-2 is prepared similarly. The substituted acetones $COMe{\cdot}CH_2{\cdot}OR$, also mostly new, are obtained in 40—95% yield by the reaction NaOR + $COMe{\cdot}CH_2Cl \rightarrow COMe{\cdot}CH_2{\cdot}OR$ [R = 2- and 4-C_6H_4Cl, 2 : 4-$C_6H_3Cl_2$, 2 : 4 : 5-$C_6H_2Cl_3$, C_6Cl_5, 2-C_6H_4Br, 4 : 2-$OPr{\cdot}C_6H_3Cl$, 4 : 2-$OBu{\cdot}C_6H_3Cl$, 2 : 4 : 5-$OAlk{\cdot}C_6H_2Cl_3$ (Alk = Et, Bu), 2 : 4 : 5-$OAlk{\cdot}C_6H_2Br_2$ (Alk = Me, Et, Pr, Bu, *iso*-C_5H_{11}). The activity of aryloxyacetic acids, aryloxyethanols, and aryloxyacetones in inhibiting germination of radish and flax seeds falls in the order given, to a negligible value for acetone derivatives. The most active of the substituted ethanols were **I**, **II** and **III**. J.A.C. Abstr.

Control of water hyacinth in Rotorua district. C. R. Taylor (*N.Z. J. Agric.*, 1955, **90**, 188—195).—Best control of this water weed was obtained by spraying twice (2—3 week interval) with an oil-based ester of 2 : 4-D in Diesel fuel (1 : 80). A. G. Pollard.

Animal Husbandry

Nutritional value of rapeseed oil meal. A review. J. M. Bell (*Canad. J. agric. Sci.*, 1955, **35**, 242—251).—A review covering information on the toxic factor, nutritional value, and current utilisation. (65 references.) P. S. Arup.

Antibiotics in animal nutrition. E. L. R. Stokstad (*Food Technol.*, 1955, **9**, 405—408).—The use of antibiotics to increase growth in animals is reviewed including the mechanism of their action; the clinical effects of continuous administration to children are summarised. (37 references.) E. M. J.

Some chemical characteristics of grass and legume silage made with sodium metabisulphite. G. Alderman, R. L. Cowan, J. W. Bratzler and R. W. Swift (*J. Dairy Sci.*, 1955, **38**, 805—810).—Only limited production of acetic and lactic acids by bacterial fermentation occurred in grass and legume silage made with $Na_2S_2O_5$ and the amounts were inversely correlated with total residual SO_3'' concn.; butyric acid production was negligible and protein breakdown considerably inhibited despite the pH being higher than normal. Preservation was apparently due to the HSO_3' ion rather than the H^+ ion. S. C. Jolly.

Fodder management on a rational basis. K. Nehring (*Acta agron. Hung.*, 1954, **4**, 347—370).—The importance of silage as an alternative to hay for winter fodder is discussed. Methods of ensilage, including the use of straw-walled silos, are compared and analytical and digestibility data are presented. Chromatographic determinations of the amino-acid distribution in various plant proteins are recorded. A. G. POLLARD.

Molybdate top dressing and animal health. I. J. Cunningham (*N.Z. J. Agric.*, 1955, **90**, 196—202).—The effects of deficiency and excess of Mo in soil and pasture are considered with special reference to N.Z. soils. Analytical data of pastures from different districts before and after top-dressing and of Mo–Cu relationships are included. A. G. POLLARD.

High quality feeding of dairy cattle: an important factor in increasing productivity. J. Herzig (*Acta agron. hung.*, 1954, **4**, 371—379).—A summary of basic principles and practical techniques in the improvement of milk yields and in the rapid fattening of young stock. A. G. POLLARD.

Metabolism of bovine semen. II. Qualitative anaërobic catabolism of ^{14}C-glucose by bovine spermatozoa. R. J. Flipse and J. O. Almquist (*J. Dairy Sci.*, 1955, **38**, 782—787).—Following the catabolism of ^{14}C-glucose by bovine spermatozoa, most of the radioactivity was found in lactic acid, although appreciable activity occurred in CO_2, volatile acids and an unidentified compound. Spermatozoa probably metabolise glucose to lactic acid, which may be further broken down to CO_2, acetate and other compounds, although, based on results with non-motile spermatozoa, CO_2 may be formed by another route. S. C. JOLLY.

Effect of methods of adding egg yolk and monosaccharides on the survival of frozen bull spermatozoa. H. D. Hafs and F. I. Elliott (*J. Dairy Sci.*, 1955, **38**, 811—815).—A significantly higher percentage of motile spermatozoa occurred in thawed semen that had been diluted prior to freezing with extender in which 25% of egg yolk was added to both the non-glycerol (*N*) and glycerol (*G*) fractions of the extender than when all the yolk was added to *N*; the 60- to 90-day non-returns were also significantly higher. Significantly higher percentages of motile spermatozoa occurred also when 1% of fructose, glucose or xylose was added either entirely to *G* or equally between *N* and *G* than when added entirely to *N*. The average percentage of motile spermatozoa was significantly higher in extenders containing fructose than in those with glucose or xylose. S. C. JOLLY.

Embryonic mortality between 16 and 34 days post-breeding in cows of low fertility. H. W. Hawk, J. N. Wiltbank, H. E. Kidder and L. E. Casida (*J. Dairy Sci.*, 1955, **38**, 673—676).—Normal embryos were recovered from the uteri of 58 and 28% of repeat-breeder cows slaughtered 16 and 34 days respectively after the first day of heat. The estimated embryonic mortality between 16 and 34 days was 51·7%. S. C. JOLLY.

Development of rumen function in the dairy calf. I. Some characteristics of rumen contents of cattle of various ages. F. W. Lengemann and N. N. Allen (*J. Dairy Sci.*, 1955, **38**, 651—656).—Development of an adult-like rumen function is gradual and begins early in the calf's life. Based on the amount of certain acids in the rumen, and on other criteria, the development is completed by about six months of age. S. C. JOLLY.

Availability of nitrogen from various ammoniated products for rumen bacteria and dairy cattle. R. F. Davis, R. H. Wasserman, J. K. Loosli, and C. H. Grippin (*J. Dairy Sci.*, 1955, **38**, 677—687).—*In-vitro* and *in-vivo* studies showed that N from ammoniated products is less readily available to rumen bacteria or to the host than is that from urea or natural protein sources. S. C. JOLLY.

Comparison of ammoniated molasses, urea and cottonseed meal as a source of nitrogen in the ration of dairy heifers. B. T. Parham, J. B. Frye, jun., B. L. Kilpatrick and L. L. Rusoff (*J. Dairy Sci.*, 1955, **38**, 664—668).—Replacement of 30% of the protein equiv. in a cottonseed meal supplement by ammoniated molasses or urea was without effect on the palatability of the feed or on wt. gains of the animals; no physiological effects occurred. More feed or energy was required to produce a unit of gain by animals receiving urea. S. C. JOLLY.

Productive value of roughages and concentrates for lactation. J. K. Loosli, R. F. Davis and R. G. Warner (*J. Dairy Sci.*, 1955, **38**, 797—804).—Replacement of part of an all-roughage ration by concentrates on the basis of equiv. total digestible nutrients is less accurate for comparing the energy value of roughages and concentrates for milk production than is replacement on an estimated net energy basis. Results do not support the theory that certain feeds contain an unidentified factor(s) that stimulates milk secretion. S. C. JOLLY.

Complementary milk and its relation to lactation. J. H. Koshi and W. E. Petersen (*J. Dairy Sci.*, 1955, **38**, 788—796).—The use of oxytocin weekly to remove complementary milk (*C*) from dairy cows had no effect on milk production. The amount of *C* decreased with progressing lactation and decreasing milk yield at normal milking. The % of *C* was independent of stage of lactation, age of animal and yield, and, because of greater consistency, was a better indicator of the animal's response to milking procedure than was the amount of *C*. There was no relation between persistency of lactation and age, the peak of production, or trend of butterfat %. Persistency was poorer in animals which reached peak production early in lactation. There was a negative correlation between persistency and % of *C*. S. C. JOLLY.

Carotene in the ration of dairy cattle. I. Influence of long periods of suboptimal carotene intake on carotene and vitamin-A values of blood, liver, and milk fat of dairy cows. J. H. Byers, P. H. Weswig, J. F. Bone and I. R. Jones (*J. Dairy Sci.*, 1955, **38**, 657—663).—Feeding Jersey and Holstein cows on suboptimal carotene rations prenatally and for long periods postnatally caused low liver-vitamin-A and -carotene values which were not increased by feeding up to 330μg. of supplementary carotene per kg. of body wt. Repeated injections of 0·25 or $1·25 \times 10^6$ i.u. of vitamin-A ester failed to increase appreciably liver vitamin A or carotene. Vitamin A and carotene values for blood plasma and milk fat from cows on suboptimal rations are also reported. S. C. JOLLY.

Additions of hay to maize silage to maximise feed intake and milk production. R. K. Waugh, H. S. Poston, R. D. Mochrie, W. R. Murley and H. L. Lucas (*J. Dairy Sci.*, 1955, **38**, 688—692).—Milk, fat, and fat-corrected milk production by cows on *ad libitum* maize silage feeding regime was increased most markedly by supplementation with 0·47 lb. of hay per 100 lb. of body wt. Optimum supplementation for max. fat-corrected milk production was calc. at $0·52 \pm 0·22$ lb. of hay. Calc. max. dry matter intake was $0·79 \pm 0·37$ lb. of hay. Body-wt. changes were not affected by the supplementation. S. C. JOLLY.

Separation and determination of acetic and lactic acids by paper partition chromatography and its application to silages. Y. Birk and A. Bond (*Analyst*, 1955, **80**, 454—457).—A method for the separation and determination of lactic and acetic acids in mixtures and silages by paper partition chromatography is described. Samples (5μl.) of unknown and standard solutions are placed near the base of sheets of prepared filter paper. One set of papers is placed in the chromatographic chamber immediately and a duplicate set is aërated at 20—30° for 5—7 hr. before being placed in the chamber. NH_4 salts of the volatile fatty acids are thus removed from the duplicate set. After ~10 hr. the papers are removed from the chamber, sprayed with an indicator solution (prep. described) and exposed to the vapour from aq. NH_3. The area of the red acid spots on a green background is measured. The total amount of acetic and lactic acids is determined from the papers placed immediately in the chamber and the lactic acid alone from those which have been aërated. Application of the method to silages is described. The solvent used in the chamber is *n*-butanol saturated with aq. NH_3. A. O. JONES.

Effect of feeding *NN'*-diphenyl-*p*-phenylenediamine to lactating dairy cows on carotene utilisation and incidence of oxidised milk flavour. R. Teichman, M. E. Morgan, H. D. Eaton and P. MacLeod (*J. Dairy Sci.*, 1955, **38**, 693—694).—Addition of 0·01% of *NN'*-diphenyl-*p*-phenylenediamine (**I**) to the rations of dairy cows increased carotene utilisation and reduced the occurrence of Cu-induced oxidised flavour in the milk. The feeding of **I** was not responsible for any of the off-flavours in the milk, had no adverse effect on the animals, and did not cause the milk to inhibit a commercial cheese starter. S. C. JOLLY.

Effect of feeding antioxidant (cacao shell) on the stability of milk fat. W. S. Mueller and K. Blazys (*J. Dairy Sci.*, 1955, **38**, 695—696).—Addition of 0·23 lb. of ground cacao shell to the ration reduced the oxidative deterioration of milk from a Jersey cow whose milk was susceptible to oxidised flavour development. S. C. JOLLY.

Use of 2 : 3 : 5-triphenyltetrazolium chloride as a test for antibiotics in milk. C. E. Neal and H. E. Calbert (*J. Dairy Sci.*, 1955, **38**, 629—633).—A simple and rapid test is described for the detection of inhibitory substances in milk using *Streptococcus thermophilus* as the test organism and 2 : 3 : 5-triphenyltetrazolium chloride as an indicator of bacterial growth. This test will detect 0·04 unit of penicillin, 0·2 μg. of aureomycin, 0·25 μg. of oxytetracycline and 4·0 μg. of streptomycin per ml. of raw milk. S. C. JOLLY.

Cobalt deficiency and vitamin-B_{12} status in cattle and sheep. F. Almasy (*Schweiz. Arch. Tierheilk.*, 1954, **96**, 630—635).—A review

of research of the past three years demonstrates that "Co deficiency" diseases are actually due to deficiency of B_{12} normally synthesised by rumen micro-organisms which need Co. Symptoms shown by stock on Co-deficient pastures are relieved by oral administration of Co or subcutaneous injection of B_{12}. (28 references.) A. G. POLLARD.

Climatic effects on foraging performance of beef cows on winter range. S. Smoliak and H. F. Peters (*Canad. J. agric. Sci.*, 1955, **35,** 213—216).—Cattalo (half- and quarter-bison breed) cows foraged on open range more frequently than did domestic breeds. Of the other factors studied (temp., windage, and supplemental feeding), temp. was by far the most important. P. S. ARUP.

Importance of milk production in the breeding of Merino sheep. J. Schandl (*Acta agron. hung.*, 1954, **4,** 381—386).—The nutritional economy of Merino sheep is discussed. Regular milking, which is essential to the profitable management of the sheep, does not affect production, has no ill-effect on the development of lambs and tends to increase the amount of milk available for suckling lambs in the following season. A. G. POLLARD.

Tests with a supplement containing penicillin for pig-feeding. W. Prock and E. Nowak (*Mitt. VersSta. Gärungsgew.*, 1955, **9,** 101—102).—Supplements of the prep. (Biocob) gave appreciably increased gains in wt. in two experiments with medium and heavy wt. pigs, but slight losses in a third experiment. P. S. ARUP.

Chemical heat regulation in the chick embryo. C. Romijn and W. Lokhorst (*Poultry Sci.*, 1955 **34,** 649—654).—An apparatus for continuously recording respiratory metabolism and temp. of the incubated egg and its application to the study of chemical heat regulation in the chick embryo are described. A. H. CORNFIELD.

Seasonal variation in the fertility, mortality and hatchability of Fayomi eggs in the subtropics. E. S. E. Hafez and G. A. R. Kamar (*Poultry Sci.*, 1955, **34,** 524—530).—Throughout the year min. fertility of Fayomi eggs (in Egypt) occurred in August and max. fertility in April. In one year the % mortality was max. in August and min. in Dec. and in another year was max. in September and min. in June. The % of pipped, emerged or hatched chicks was low in June and high in March, April and Sept. Fertility was affected more by temp. than by R.H. Hatchability was affected by R.H. independently of temp. A. H. CORNFIELD.

Effect of adrenaline on oviduct motility and egg production in the fowl. A. H. Sykes (*Poultry Sci.*, 1955, **34,** 622—628).—Injections of adrenaline (0·00025—0·010 g. per bird) delayed oviposition in the hen and lowered subsequent egg production in proportion to the amounts injected. Doses too small to delay oviposition were effective in lowering egg production. *In vitro* studies of the activity and reactivity (to adrenaline and pituitrin) of the oviduct are also reported. A. H. CORNFIELD.

Progesterone-induced unseasonable moult in Single Comb White Leghorn pullets. J. L. Adams (*Poultry Sci.*, 1955, **34,** 702—707).—Rapid moulting of laying birds occurred on treatment with progesterone (two weekly injections with 0·013 g. for 9·5 weeks, or the same treatment for 5·5 weeks with a terminal dose of 0·04 g.). Moulting ceased about two weeks after the final dose. Egg production ceased immediately after the first injection and started again about 25 days after the final injection. Body wt. was slightly reduced by the treatments but returned to normal within 2—4 weeks after discontinuing the treatments. A. H. CORNFIELD.

Effects of progesterone on growth and sexual development in Single Comb White Leghorns. T. W. Fox (*Poultry Sci.*, 1955, **34,** 598—602).—Weekly intramuscular injections of progesterone (0·002—0·016 g. per bird) had no significant effect on body wt. or shank length at 12 weeks of age, although the variability in these values were increased. Comb index (height × length) of males and testis size were reduced by the treatments. A. H. CORNFIELD.

Force moulting (interruption of egg laying) in White Leghorn hens by the use of Enheptin (2-amino- 5-nitrothiazole). J. A. Pino (*Poultry Sci.*, 1955, **34,** 540—543).—Egg production of 20-month-old birds fell rapidly to zero on administration of Enheptin (0·1% in the feed for one week followed by 0·05% for another week). On discontinuing the treatment egg production increased gradually and after six weeks was at a higher level than that of controls. The treatment caused profuse moulting of all birds. The pattern of egg production of Enheptin-treated birds was similar to that of birds force-moulted by feed restriction. A. H. CORNFIELD.

Rate of daily feed consumption, the daily maximum protein anabolism, and their importance concerning the evaluation of feeding experiments with antibiotic supplements. H. W. Hohls (*Poultry Sci.*, 1955, **34,** 717—724).—A review of published work indicates that increased body wt. gains due to antibiotic supplementation were affected by feed consumption and particularly by protein consumption in poultry. Penicillin had no effect on growth when the daily protein consumption exceeded the daily protein anabolism. The importance of noting protein consumption in tests with antibiotics is stressed. A. H. CORNFIELD.

Effects of selecting for high and low thyroidal response to thiouracil feeding in New Hampshire chickens. W. E. Shaklee and C. S. Shaffner (*Poultry Sci.* 1955, **34,** 572—577).—Selection for high and low response in thyroid gland wt. at four weeks of age to the feeding of thiouracil (0·2% in the diet) to chicks resulted in a five-fold difference in the thyroid wt. of the treated birds after three generations of selection. There were no significant differences between birds of the high and low lines in egg production, fertility, hatchability, incubation period, growth or feathering rate, or mortality. A. H. CORNFIELD.

Effect of restricted feeding on several genetically controlled characteristics in the fowl. A. J. Schneider, B. B. Bohren and V. L. Anderson (*Poultry Sci.*, 1955, **34,** 691—702).—Birds on a restricted low-protein diet showed retarded growth in comparison with those receiving a full high-protein diet. Sexual maturity was retarded by 2—4 weeks in the restricted group. Mortality during growth was similar for both groups, whilst adult mortality was lower for the restricted group. Total egg production was similar for both groups; the restricted group laid fewer eggs in the early part, but more eggs in the later part, of the laying period than did the high-protein group. The restricted group produced more eggs of hatchable size and showed slightly higher fertility and hatchability than did birds receiving the full ration. A comparison of a high- with a normal-protein laying ration showed that the latter was superior with respect to fertility and hatchability of fertile eggs and total eggs set. Both rations gave similar results with respect to body wt., viability, egg size and egg production. A. H. CORNFIELD.

Barley in high-efficiency broiler rations. I. Influence of methionine grit and stabilised animal fat on efficiency of utilisation. G. H. Arscott, L. E. Johnson and J. E. Parker (*Poultry Sci.*, 1955, **34,** 655—662).—The use of barley up to 25% of the grain component (13—15% of the total ration), replacing maize, in high-energy broiler rations had no adverse effect on growth to 10 weeks. Growth depression occurred when 50—100% of the grain component of the ration was made up of barley. Body pigmentation decreased with increasing % of barley in the ration. Addition of 0·05% of methionine, with or without granite grit, to rations containing barley had no effect on growth or feed efficiency. Addition of 4—8% of stabilised prime tallow to rations in which barley constituted 25—50% of the grain component improved feed efficiency to the extent that the rations compared favourably with all-maize rations. A. H. CORNFIELD.

Chicken feather meal as a feed supplement. O. H. M. Wilder, P. C. Ostby and B. R. Gregory (*Poultry Sci.*, 1955, **34,** 518—524).—Excellent growth of chicks and high feed efficiency to 6—8 weeks of age was obtained when feather meals were added to practical diets (containing soya-bean oil and lucerne meals and maize) to supply 2·4% of the protein of the diets. When feather meal supplied 6·2% of the protein of the diets good growth was obtained only when 0·4% of lysine was supplied. Blood meal and meat and bone scraps were satisfactory sources of essential amino-acids needed for supplementing feather meal diets. A. H. CORNFIELD.

Protein requirement of broilers as influenced by antibiotics. J. W. West and J. E. Hill (*Poultry Sci.*, 1955, **34,** 628—634).—Tests with diets containing protein levels ranging from 16 to 24% showed that 18% of protein in the diet was sufficient for optimal growth and feed efficiency to nine weeks of age when aureomycin, Terramycin, bacitracin (10 g.) or procaine penicillin *G* (2 g. per ton of feed) was supplied. When no antibiotic was supplied a protein level of ≮20% was required for optimal growth and feed efficiency. The antibiotics exerted a sparing action on the protein requirements of the birds. A. H. CORNFIELD.

Cottonseed meal as a substitute for soya-bean oil meal in poultry rations. J. W. West (*Poultry Sci.*, 1955, **34,** 547—553).—Degossypolised solvent cottonseed meal replaced up to 80% of the soya-bean oil meal in broiler and turkey starter rations without adverse effects upon growth or feed efficiency. Complete substitution of soya-bean oil meal with cottonseed meal resulted in reduction in growth rate of starting turkeys as well as in that of broilers in about half of the tests. There were indications of a slight although consistent complementary effect when the two meals were supplied together. The presence of cottonseed meal in the feed had no effect on uniformity carcass finish, or moisture content of the litter. A. H. CORNFIELD

Effects of various processing methods on the value of cottonseed meals as an amino-acid source for chickens. C. R. Grau and P. A. Zweigart (*Poultry Sci.*, 1955, **34**, 724—728).—Cottonseed meals produced by several different methods (screw-pressed, prepressed solvent-extracted, and hydraulic pressed at various temp.) were all satisfactory sources of amino-acids for chicks when fed to supply the sole protein of the diets. In general, somewhat better growth was obtained with meals cooked below 93·3°. Growth rate was correlated with 0·02N-NaOH-sol. N in the meals. A. H. CORNFIELD.

Unidentified chick growth factors. I. Purified assay diet and crude supplement response. C. F. Petersen, A. C. Wiese and A. R. Pappenhagen (*Poultry Sci.*, 1955, **34**, 673—678).—A purified diet which was very suitable for studying unidentified growth factors in added materials is described. New Hampshire chicks (obtained from breeder hens receiving a ration containing herring fish meal and maintained on deep litter) were deficient in an unidentified factor(s) which was present in herring fish meal, sardine fish solubles, a 50% lactose dried whey product, Biopar "C" and a defatted liver residue (Abbott). A. H. CORNFIELD.

Unidentified chick growth factors. A. A. Camp, H. T. Cartrite, J. H. Quisenberry and J. R. Couch (*Poultry Sci.*, 1955, **34**, 559—566).—An unidentified chick growth factor was present in dried whey and fermented whey and another factor in fish solubles and an antibiotic fermentation product. Hydrolysed whey either contained a factor different from these two or increased the energy in the diet resulting in a significant increase in growth rate of chicks when added to a diet containing either fish solubles, dried whey, or fermented whey. A. H. CORNFIELD.

Unidentified mineral required by the chick. A. B. Morrison, M. L. Scott and L. C. Norris (*Poultry Sci.*, 1955, **34**, 738—740).—Chicks from hens fed a diet which depleted their progeny of unidentified growth factors made a considerable growth response to a mixture of five unidentified growth factor supplements (distillers' dried solubles, fish solubles, grass juice, dried whey product and penicillin mycelium meal). The AcOH-neutralised ash of the mixed growth factor supplements promoted a growth response of approx. 50% of that obtained from the mixed supplements. Addition of all known essential trace minerals or alteration of the Ca and P contents of the diet did not affect growth. The mixed supplements probably contain an unidentified mineral essential for chick growth apart from containing factors of org. nature. There was a consistent, although not as great, response to both the mixed supplements and its neutralised ash when non-depleted commercial chicks were used. A. H. CORNFIELD.

Utilisation of fats of different melting points added to broiler feeds. D. S. Carver, E. E. Rice, R. E. Gray and P. E. Mone (*Poultry Sci.*, 1955, **34**, 544—546).—Addition of tallow, hydrogenated tallow, or hydrogenated tallow fatty acids (each at the 3% level) to the diet of chicks from 0—4 weeks of age had no effect on growth rate. Feed efficiency was improved only by tallow. There was 81—100% utilisation of tallow by the birds, whilst the other materials were utilised relatively poorly. The chicks hydrolysed 90% of the fats supplied; lack of digestion did not account for poor absorption of hydrogenated fats. A. H. CORNFIELD.

Stability of vitamin A in mixed feeds and premixes. B. L. Reid, H. K. Daugherty and J. R. Couch (*Poultry Sci.*, 1955, **34**, 603—608).—When vitamin A in the form of fish liver oil was mixed with a no. of rations there was considerable loss of vitamin A even after one month storage at room temp. The loss was much less when the rations were stored at 5·5°. When the vitamin was mixed in the form of a stabilised dry concentrate there was little loss of vitamin A after four months storage at room temp. Results of chemical and biological methods of assessing vitamin A agreed closely. A. H. CORNFIELD.

Toxicity of treated maize seed in rations for chicks. C. W. Ackerson and F. E. Mussehl (*Poultry Sci.*, 1955, **34**, 728—729).—The presence of 0·043% of Orthocide (75%) in the diet of chicks reduced their growth rate slightly to 21, but not to 28 days of age. The presence of 0·067% of Arasan (50%) in the diet considerably reduced chick growth rate to 25 days of age but on withdrawing the treatment the growth rate returned immediately to normal. The amounts of seed protectants present in the diets were of the order of those likely to be present through using maize treated with these materials. A. H. CORNFIELD.

Effect of sodium chloride upon the apparent viscosity of egg yolk, egg white and whole egg magma. R. Jordan and E. S. Whitlock (*Poultry Sci.*, 1955, **34**, 566—571).—Treatment of whole egg magma (blended white and yolk) and egg white with 1—5% of NaCl reduced slightly the apparent viscosity of the products, whilst treatment of yolk greatly increased the apparent viscosity. The translucency and orange colour of the whole egg magma and egg yolk were increased by the treatments. A. H. CORNFIELD.

Egg white lysozyme. III. Effect of pH on the lysozyme-ovomucin interaction. O. J. Cotterill and A. R. Winter (*Poultry Sci.*, 1955, **34**, 679—686).—Increasing the pH from 7·2 to 10·4 decreased the intensity of the *in vitro* interaction of lysozyme with ovomucin, especially when ovomucin was supplied by thick white. The pptn. interaction between lysozyme and ovomucin practically disappeared at approx. pH 9·0—9·5; this is the pH at which enhanced rate of egg white thinning occurs. A. H. CORNFIELD.

Artificial insemination in turkey breeder flocks. C. E. Stotts and M. I. Darrow (*Poultry Sci.*, 1955, **34**, 508—518).—Studies with 21 Broad Breasted Bronze turkey flocks located in various parts of the U.S. showed that the use of artificial insemination as a supplement to natural mating improved fertility in healthy flocks having a natural fertility of 85% or less. Flocks inseminated during the early part of the laying season exhibited only a slight decline in fertility up to 30 days after insemination, whilst flocks inseminated during the last half of the laying season showed a marked drop in fertility after 20 days. The use of artificial insemination as a supplement to natural mating late in the season on previously naturally mated flocks raised fertility to approx. the peak-season level. Insemination lowered egg production in most cases, but this effect was confined to the day of and that following insemination. A. H. CORNFIELD.

Relation of frequency of collection to amount of semen obtained from turkey males. F. W. Lorenz, N. E. Wilson and V. S. Asmundson (*Poultry Sci.*, 1955, **34**, 634—639).—The total vol. of semen collected per week from male turkeys increased with frequency of collections up to seven collections per week. Vol. per sample decreased gradually during the first week of daily collections and became stabilised after five or six collections. Frequency of collection had no effect on semen concn. The no. of sperms per sample was not affected by frequency of collection except where very low vol. of semen were obtained due to prolonged daily collections. A. H. CORNFIELD.

Ambient temperature as a factor in turkey reproduction. II. Effect of artificially lowered air temperature on the breeding activity of males in late spring and in summer. I. L. Kosin and M. S. Mitchell (*Poultry Sci.*, 1955, **34**, 499—505).—The decline in fertility of male turkeys which normally occurred during May—July was largely prevented by transferring the birds to cooled or partially shaded pens from 9 a.m. to 4 p.m. daily. Birds subjected to the cooled environments maintained a lower body temp. than did those under natural conditions. A. H. CORNFIELD.

Shade requirement of growing turkeys. W. O. Wilson, W. H. Edwards, T. L. Plaister, J. Hillerman and A. Woodard (*Poultry Sci.* 1955, **34**, 505—508).—Varying the amount of shade from 0 to 2·6 sq. ft. per bird had no consistent effect on growth rate, feed consumption, mortality, or incidence of pendulous crops from 12 to 26 weeks of age. On hot days water consumption increased with decreasing amount of shade. Rectal temp. of the birds was inversely related to the amount of shade only when air temp. was high. A. H. CORNFIELD.

Effects of a detergent in the diet of range turkeys. H. J. Almquist and J. B. Merritt (*Poultry Sci.*, 1955, **34**, 740—741).—Addition of 0·10—0·15% of Oronite D-40 (a Na alkyl-arylsulphonate) to the diet of range turkeys improved wt. gains very slightly but definitely improved feed efficiency, particularly of females. The effects of the treatment were most noticeable when birds were consuming relatively large amounts of whole grain, indicating that the detergent may have improved the digestion of the whole grain. A. H. CORNFIELD.

Dehydrated lucerne meal, condensed fish solubles, distillers' dried solubles, and dried whey as supplements to an all-vegetable protein turkey laying diet. R. L. Atkinson, T. M. Ferguson, J. H. Quisenberry and J. R. Couch (*Poultry Sci.*, 1955, **34**, 730—735).—Dehydrated lucerne meal and condensed fish solubles were good sources of the unidentified factor(s) required to maintain high fertility and hatchability of turkey eggs from dams receiving all-vegetable diets over a long period. Distillers' dried solubles was a somewhat poorer source of the factor(s) present in condensed fish solubles, whilst dried whey contained negligible amounts of the factor(s). A. H. CORNFIELD.

Organoleptic and histological characteristics of fresh and frozen stored turkey fed high-density and low-density rations. G. E. Goertz, G. E. Vail, D. L. Harrison and P. E. Sanford (*Poultry Sci.*, 1955, **34**, 640—648).—Roasted turkeys raised on high-density (low-fibre) rations were scored slightly higher with respect to aroma and flavour than were birds raised on low-density (high-fibre) rations. The former contained larger amounts of intramuscular fat and had lower juiciness grading and smaller % total cooking losses than did

the latter. Tenderness gradings were similar for birds on both types of ration. Steaks from both types of birds were still acceptable after eight months storage at −17·8°. A. H. CORNFIELD.

Methyl bromide for sterilising poultry litter. S. A. Edgar and D. F. King (*Poultry Sci.*, 1955, **34**, 595—597).—Laboratory tests showed that *Aspergillus fumigatus* spores, *Ascaridia galli* eggs and *Eimeria tenella* oocysts were killed by applying MeBr at the rate of 1 ml. per quart of space or at 1 lb. per 100 sq. ft. of floor space. Under commercial conditions it is possible that lower concn. may be equally effective. A. H. CORNFIELD.

Foot-rot of sheep. W. I. B. Beveridge (*Univ. Federation Anim. Welfare*, 1955, *Pamphlet*, 11 pp.).—A review of the nature of the disease, its effects, and means of eradicating it. E. G. BRICKELL.

Chronic respiratory disease of chickens. J. F. Crawley and J. E. Fahey (*Poultry Sci.*, 1955, **34**, 707—716).—A plan for the control of the disease is outlined. A. H. CORNFIELD.

Therapeutic use of antibiotics for chronic respiratory disease in three laying flocks. M. S. Cover (*Poultry Sci.*, 1955, **34**, 686—690).—The course of chronic respiratory disease, as indicated by external symptoms, egg production, and mortality, in three infected flocks was unaffected by subcutaneous injections of Terramycin in oil or streptomycin–penicillin paste, intramuscular injections of bicillin–streptomycin, or addition of Terramycin to the drinking water. A. H. CORNFIELD.

Serological analysis of seven strains of pleuropneumonia-like organisms from air sac infection in poultry. E. M. Gianforte, E. L. Jungherr and R. E. Jacobs (*Poultry Sci.*, 1955, **34**, 662—669).—Seven strains of pleuropneumonia-like organisms isolated from so-called air sac infection in poultry and representing different avian species and geographic areas were examined serologically. The results indicate that serological testing of birds for air sac infection may be possible. A. H. CORNFIELD.

Dietary aureomycin and immune response to Newcastle disease and infectious bronchitis. F. S. Markham, R. J. Price, K. Seeger and R. White-Stevens (*Poultry Sci.*, 1955, **34**, 554—559).—Three broiler flocks reared under field conditions were supplied with aureomycin (10—300 g. per ton of feed) from the time of hatching. The treatments had no significant effect on the ability of the bird to develop a normal immune response to Newcastle disease or infectious bronchitis induced by vaccination. A. H. CORNFIELD.

Propagation of the virus of visceral lymphomatosis in embryonated eggs. R. F. Gentry and B. R. Burmester (*Poultry Sci.*, 1955, **34**, 669—672).—The virus of RPL-12 visceral lymphomatosis was successfully carried through five passages in embryos. The potency of the material after various passages is reported. A. H. CORNFIELD.

Leucine and fowl ascarids. B. B. Riedel (*Poultry Sci.*, 1955, **34**, 587—589).—Varying the amount of leucine in the diet (from 1·31 to 1·65 lb. per 100 lb. of feed) of chickens had no significant effect on the resistance of the birds to *Ascaridia galli* infection. A. H. CORNFIELD.

Growth-curve studies of chick embryo-propagated infectious bronchitis virus. S. B. Hitchner and P. G. White (*Poultry Sci.*, 1955, **34**, 590—594).—The growth curves are presented and discussed. A. H. CORNFIELD.

Herbicidal compositions. E. I. du Pont de Nemours and Co. (B.P. 727,906, 4.6.52. U.S., 11.6.5, addition to B.P. 691,403 of 28.11.50).—Herbicidal compositions are claimed which contain (*a*) a herbicidally active compound selected from the group NH_4 sulphamate; dinitrophenols; pentachlorophenates; 2 : 4-dichloro- and 2 : 4 : 5-trichloroacetic acid, their salts or amides; or NH_4, Na, K or Ca salts of HCNO, HCNS or trichloroacetic acid, and (*b*) a herbicidal compound X·NH·CO·NRR′, where X is a phenyl group which may contain 1—4 substituents such as halogen, C_{1-8}-alkyl, or -alkoxy, R is a C_{1-3}-alkyl group and R′ is H or alkyl (1—3 C atoms) (preferably R = R′ = Me, X = phenyl, chlorophenyl or 3 : 4-dichlorophenyl). Such mixtures are synergic and are economically more effective for the control of weeds than are the herbicidal components of the mixture when employed separately. Examples are given of powders dispersible in water, and oil–water powders, etc. Results obtained in herbicidal tests are given to support the claims. M. DAVIS.

Substituted aralkyl-polyalkylene-polyamino-polyacetic acids and salts thereof. F. C. Bersworth (B.P. 721,640, 26.9.52).—Substituted aralkyl-polyalkylene-polyamino-polyacetic acids and salts corresponding to the formula $C_6X_5\cdot[CH_2]_z\cdot[N(CH_2\cdot CO_2M)\cdot A\cdot]_n\cdot N(CH_2\cdot CO_2M)\cdot A\cdot N(CH_2\cdot CO_2M)\cdot[CH_2]_z\cdot C_6H_5$, where *n* is 1, 2, 3 or 4; *z* is 1, 2, 3, 4 or 5; X is alkyl, hydrogen, halogen, hydroxyl or alkoxyl, one to three of the X groups of each phenyl nucleus being other than hydrogen; A is ethylene, propylene or trimethylene; and M is hydrogen, alkali metal, ammonium or a substituted ammonium group, are prepared. The compounds are water-soluble bactericides and fungicides and have a strong sequestering power for metal ions (particularly the transition metals) in aq. solution. The prep. of bis-(2 : 6-dichloro-4-hydroxybenzyl)diethylenetriaminetriacetic acid is described. J.A.C. ABSTR.

2.—FOODS

Chemical distinction between proteins of wheat and rye. I. Behaviour of the proteins with sodium hypochlorite. M. Rohrlich, G. Adler and O. Kramm (*Z. LebensmittUntersuch.*, 1955, **102**, 85—97).—A similar structure is probably assignable to the extracts obtained from wheat with the solvents aq. EtOH, water, aq. salts, and aq. acids, since the rates (although not the amounts) of CO_2 and N_2 evolution with Na hypochlorite are practically identical. For wheat, the vol. of gases evolved when aq. 2% NaCl is used as solvent are greater than when 2% KCl is used; for rye this difference is not observed, and, moreover, the amount of extract obtained is approx. twice that obtained from wheat. Comparisons between the behaviour of N-acetic acid extracts from wheat and rye also point to structural differences between the proteins of the two grains. (41 references.) P. S. ARUP.

Improvement of maize gluten. E. Lindemann (*Stärke*, 1955, **7**, 112—116).—Increased protein content of gluten, remaining after the extraction of starch from maize, was obtained in two ways: (*a*) extraction of protein from wet gluten by dissolution with NaOH and K sulphide, followed by pptn. of the neutralised solution at the isoelectric point (pH 4·6); this method increased the protein content of gluten from ~68 to 90% (w/w), but is not considered economical; and (*b*) treatment of wet gluten at 40° and pH 6 with amylolytic enzymes for 15 min.; the final protein content was 81%, which was raised to 88% by solvent extraction of fats; this process is recommended for plants which have no modern centrifugal separators. E. DUX.

Manufacture of maize gluten. A. Böyng (*Stärke*, 1955, **7**, 119—123).—An illustrated description of modern gluten extraction and concn. practice, using centrifugal separators, is given, with flowsheets. Several different nozzle separators are described. If the separator is used with built-in washing water supply, protein values in the gluten of >70% are achieved. The concentration of gluten by re-cycling is dealt with. E. DUX.

Selective degradation of wheat gluten. L. Wiseblatt, L. Wilson and W. B. McConnell (*Canad. J. Chem.*, 1955, **33**, 1295—1303).—Wheat gluten is hydrolysed by the method of Eliott (*Biochem. J.*, 1952, **50**, 542) to *N*-acetyl peptides. This method is believed to hydrolyse peptide bonds selectively at the amino-groups of serine, but introduces sulphonic acid groups. Hydrolysis with 1% HCl in methanol yields a de-acetylated peptide (**I**) sol. in methanol, and a still-acetylated peptide (**II**) insol. in methanol. Nearly all the serine appears at the amino termini of the peptides. Both electrophoresis on paper and by the moving-boundary method show that **I** and **II** are remarkably homogeneous, though treatment with alkali modifies the electrophoretic behaviour. Osmotic pressure measurements on **I** indicate a mol. wt. of approx. 20,000. Terminal group estimates show several *N*-terminal serine residues per mol. There is evidence of strong association or cross-linking between peptide chains. (23 references.) A. B. DENSHAM.

Manufacture of wheat starch by continuous process. W. Hönsch (*Stärke*, 1955, **7**, 107—112).—An illustrated description of modern Australian wheat starch manufacture is given, with flowsheets. Flour is mixed with water in a batter mixer to a dough and sprayed with water to extract gluten, the latter being dried in a flash drier. Starch extraction is carried out in two-stage centrifugal separators, followed by flash drying. The process is continuous, producing ~430 kg. of cornflour, of 99·6% purity, per hr. The yield is 61·3%, the albumin content 0·2% and the fibre content zero. E. DUX.

Starch hydrolysates and glucose reversion mixtures. A. S. Spriggs (*Dissert. Abstr.*, 1955, **15**, 709—710).—The carbohydrate constituents of hydrol, the residual syrup remaining after the crystallisation of D-glucose in the commercial production of glucose by acid hydrolysis and of complex mixtures formed by the action of aq. HCl on D-glucose, were studied by column and paper chromatography and by radio-isotopic dilution methods. In hydrol, the following were found: D-glucose (65%), gentiobiose (9%), *iso*maltose (9·7%), 5-β-D-glucopyranosyl-D-glucose (**I**) (0·9%) and β-cellobiose (0·4%) with 3·9% of unidentified higher oligosaccharides. The following were identified in the products of action of aq. HCl on D-glu-

β-maltose hydrate (~1·5%) αα-trehalose dihydrate (~1%), *iso*-maltose (~5%), gentiobiose (~4%), **I** (~0·1%), β-cellobiose (~1·5%), and higher oligosaccharides (~7%). J. S. C.

Polyfructosans. XLII. Phlein and its enzymic structure. H. H. Schlubach and H. Lübbers (*Liebigs Ann.*, 1955, **594**, 41—58).—Artificial mixtures of "phlein," a collective term for the homologous series of polymeric fructofuranose ring compounds, are separated by repeated fractionation from chloroform solution by the addition of ethanol to their acetylated deriv., which are subsequently de-acetylated and further characterised by a determination of their sp. rotation. It is assumed that a pure phlein 15 has a rotation of $[\alpha]_D^{20} = -34{\cdot}5°$. The degree of polymerisation is determined by a graphical osmotic method. Enzyme activity was studied by dissolving phlein in water and an acetate buffer at pH 4·7 and adding invertase concentrate; the degree of degradation was followed polarimetrically until a 45% change was reached. The phlein obtained by pptn. from the solution had a rotation of −41·4° and a reduction value of 0·5%. Similar degradations were made at pH 7. G. R. WHALLEY.

Lipoxidase activity of wheat. J. A. Blain and J. P. Todd (*J. Sci. Food Agric.*, 1955, **6**, 471—479).—The application of an assay based on destruction of carotene to the evaluation of lipoxidase activity in wheat is described. A carotene–linoleate system developed for the assay of soya extracts is used. This assay operates at a pH at which the primary substrate, linoleate, and the enzyme are in different phases, and any natural surface-active factors occurring in crude extracts of lipoxidase may affect dispersion and reaction interface. Certain characteristics of the assay as established for soya are reassessed. The distribution of lipoxidase in the wheat berry is discussed. (15 references.) E. M. J.

Bread-making quality of some American wheat grown in Toledo. Vicente Martin Vargas (*Bol. Inst. Invest. agron. Madr.*, 1955, **15**, 72—93).—The bread-making qualities of a no. of American wheats grown in Spain have been compared by the Brabender farinograph and fermentograph, the Chopin alveograph and baking tests on 75% extraction flours. F. R. PAULSEN.

Flour- and bread-quality of wheat imported during 1954. Tomas De la Vega and Fernando Silvela (*Bol. Inst. Invest. agron. Madr.*, 1955, **15**, 95—158).—Some 688 million kg. of wheat were imported into Spain during 1954, from U.S.A., Turkey and Portugal. These three groups are compared on a basis of analysis, fermentation time, flour extraction, etc., while statistics are given for the ships, ports, periods of year, loads, etc. which were involved. F. R. PAULSEN.

Influence of flour treated with potassium bromate and ammonium persulphate upon dough produced by various methods. O. Doose and K. Wolter (*Trans. Amer. Ass. Cereal Chem.*, 1955, **13**, 130—147).—Bread defects due to overtreatment of dough with these chemicals resemble those produced by the overmaturing of dough, and are due to acceleration of maturing. Comparatively large doses of K bromate give good results, as regards loaf vol. and structure, only from young doughs, with low yeast content. Dough rendered defective by overtreatment, or by overmaturing (without K bromate) can be made usable by remixing with small amounts of water and sugar. The relations between dough and loaf quality and physical factors (notably the state of combination of the water) are considered. P. S. ARUP.

Precise gassing-power unit [for flour testing]. I. Hlynka and V. Martens (*Trans. Amer. Ass. Cereal Chem.*, 1955, **13**, 147—151).—Variability in results obtained in gassing-power measurements is largely due to lack of uniformity in construction of the apparatus. A unit of standard dimensions (described) consisting essentially in a brass cup with a collar to seat the lid, a lid carrying a pressure gauge and release-valve, and a clamp has given satisfactory results over a long period. Directions are given for the standardisation and operation of the unit, and for mixing the dough under standard conditions. P. S. ARUP.

Importance of oxidation on use of soya-bean flour with high-extraction wheat flours. C. W. Ofelt and Allan K. Smith (*Trans. Amer. Ass. Cereal Chem.*, 1955, **13**, 122—129).—The use of 6–8 mg. per 100 g. of K bromate counteracts the deleterious effects on dough-handling properties and bread characteristics of the admixture of 5% of soya-bean flour with 83—90% extraction wheat flours, baked without sugar or shortening. The response to bromate is more pronounced with high-protein than with soft wheats. The effect of soya-bean flour used alone is least with the high-protein flours. P. S. ARUP.

Reaction of present-day bread-flour to mould-enzymes. G. Bergh (*Bakkerswereld*, 1954/55, **15**, No. 24, Repr., 8 pp.).—Previously reported improvements in bread quality obtained with the addition of two enzymic prep. in the flour (cf. *Chem. Weekbl.*, 1953, **49**, 657) are maintained in the present-day Dutch flour which contains a greatly increased proportion of native wheat. The effects of the prep. as regards improvement in quality are not primarily connected with their amylolytic or proteolytic properties. P. S. ARUP.

Daily bread in America. II. Continuous mixing and kneading as means of simplifying and shortening bakery production. H. M. R. Hintzer and G. J. Langenberg (*Bakkerswereld*, 1954/55, **15**, No. 27, 28, Repr., 14 pp.; cf. *ibid.*, No. 4, 5, 6).—A review covering the principal systems in use. P. S. ARUP.

Yeast-salt method [in bread-making]. F. Bothma (*Misset's Bakkerswereld*, 1954/55, **15**, No. 47, Repr., 4 pp.).—The advantages claimed for the method of adding yeast to the dough in the form of a suspension in aq. NaCl are not obtained under the conditions of Dutch bakery practice. P. S. ARUP.

Metabolism of radioactive ethanol in baker's yeast. G. Milhaud and J.-P. Aubert (*C. R. Acad. Sci., Paris*, 1955, **240**, 2178—2179).—Ethanol-1,2-^{14}C was used to study the metabolism of ethanol in yeast. The radioactivity was largely concentrated in the compounds formed in the citric cycle and their immediate deriv. The formation from ethanol in yeast was, for the first time, observed for phosphopyruvic acid, phosphoglyceric acid, phosphates of hexoses and trehalose, thus establishing the reversibility of the glycolysis. It was also established that ethanol, although a product of the normal metabolism of yeast by breakdown of glucose, is simultaneously utilised with glucose and that it gives rise, in these conditions, to compounds specifically related to the tricarboxylic (citric) cycle. J. S. C.

Reversibility of glycolysis in baker's yeast studied with ethanol-2-^{14}C. J.-P. Aubert and G. Milhaud (*C. R. Acad. Sci., Paris*, 1955, **240**, 2451—2453).—Trehalose (**I**), 3-phosphoglyceric acid (**II**), phosphoenolpyruvic acid (**III**) and alanine (**IV**), formed by yeast from ethanol-2-^{14}C were subjected to degradation and the relative radioactivity in the C-atoms studied to determine their mode of formation. **I** synthesised by yeast from ethanol is formed by the condensation of fragments derived from the C_3-atom of glucose. **II, III** and **IV** appear to have a double origin: carboxylation of a C_2-fragment, and formation from an intermediate of the citric cycle. (10 references.) J. S. C.

Cooked potatoes in bread. F. Bothma (*Bakkersvakblad*, 1955, **14**, No. 29, Repr., 4 pp.).—The incorporation in bread of 5—15% of cooked potatoes does not improve its retention of softness, but causes deterioration in this and other respects. P. S. ARUP.

Review of bread staling research. W. G. Bechtel (*Trans. Amer. Ass. Cereal Chem.*, 1955, **13**, 108—121).—A review with 81 references. P. S. ARUP.

Working in of returned bread. F. Bothma (*Bakkersvakblad*, 1955, **14**, No. 28, Repr., 4 pp.).—The adverse effects on quality indices of working in stale bread with fresh dough are shown in tabulated results based on experiments in which the stale bread was first homogenised to a paste with water, and then added to the fresh dough in various proportions and under comparable conditions. In general, the proportions of 5—6% of stale bread without crust, or 3—4% with crust cannot be exceeded without undue deterioration in quality. The opinion that the addition of stale bread promotes the retention of softness is not confirmed. P. S. ARUP.

Fermentation industries. J. Mejane (*Monde industr.*, 1955, **81**, No. 512, 5—22).—The main types of fermentation are briefly described and their industrial applications reviewed. The industries and processes mentioned are: distilleries, yeast manufacture, brewing, glycerol manufacture, bread, vinegar, sauerkraut, cattle fodder, lactic acid, retting of textiles, and manufacture of butter and cheese. J. S. C.

[Analysis of phosphates.] New titrimetric determination of pyro- and orthophosphates. T. Kato, Z. Hagiwara, R. Shinozawa and S. Tsukada (*Technol. Rep. Tohoku Univ.*, 1954, **19**, 93—103).—An accurate analytical method is established for the determination of pyro- and ortho-phosphates in commercial substances. Pryophosphate is determined by pptn. with Zn acetate, dissolving the ppt. in ammonia and titrating with EDTA using Solochrome black as indicator; quant. pptn. of Zn pyrophosphate occurs in the pH range 3·8—3·9. Ortho-phosphate is determined by pptg. Mg NH_4 phosphate, dissolving in dil. HCl, adding a measured excess of EDTA and back-titrating with standard $MgCl_2$ at 30—40°. J.A.C. ABSTR.

Improvement of the operation of sugar factories by automatic control. V. Broida (*Génie chim.*, 1955, **73**, 153—162; **74**, 19—26).—The application of instrumentation and automatic controls in the following stages of beet sugar manufacture are described: juice

heating, diffusion (battery and Olier continuous type), first and second carbonatation (discontinuous), evaporation, sugar boiling. J. S. C.

The control of molasses in sugar factories and refineries. G. Pidoux (*Industr. agric. aliment.*, 1955, **72**, 395—401).—The various formulae and calculations which have been proposed for evaluating the extraction of molasses are described and discussed. It is pointed out that molasses is composed not only of the residual syrup from the C stage crystallisation but also contains fine sugar crystals which have passed through the mesh of the centrifugal baskets, either as a result of imperfect boiling at the C stage or mechanical imperfections. A method of determining the % sugar crystals in molasses is described, based on determining the Brix of the molasses direct with a refractometer and then diluting to a known proportion with hot water to dissolve the crystals, after which the Brix is again determined refractometrically. The various conceptions of "purity" in molasses are defined and compared and their use is illustrated by an actual example drawn from a cane-sugar refinery. J. S. C.

Preliminary investigations into the chemistry of sulphitation. A. Carruthers, J. F. T. Oldfield and A. E. Wootton (*Brit. Sugar Corp. 8th Tech. Conf.*, 1955, 36 pp.).—An hypothesis is presented that invert sugar is degraded to form three intermediate compounds A, B and C; C reacts with SO_3'' irreversibly to give a colourless compound G; A undergoes an irreversible reaction to give a coloured compound D. B may react with SO_3'' to give a colourless compound F, the reaction being reversible, or B may react irreversibly to form a coloured compound E. Before any sulphite is available for prevention of colour, C must have reacted to saturation. SO_2, added before colour is produced, must be present in excess of a certain min. quant. dependent on the extent of invert destruction. Sug. Ind. Abstr. (E. M. J.).

Activity of thermophilic bacteria in sugar-beet diffusion systems. A. Carruthers and J. F. T. Oldfield (*Brit. Sugar Corp. 8th Tech. Conf.*, 1955, 47 pp.).—Factors affecting the intensity of thermophilic activity in the diffusion system are discussed and experimental tests on variation of these factors are described. At Colwick bacterial activity is reduced by injecting 3 gal. of formaldehyde into cell 14 every four hours (a 0·005% dose); at Brigg, where there are two R.T. diffusers, 4 gal. are injected into cell 16 every eight hours. Lactic acid is the chief acid formed and this induces additional corrosion comparable to that induced by HCl or H_2SO_4. Recommendations are made for conditions of temp. pH and plant design in the diffusion system. Sug. Ind. Abstr. (E. M. J.).

Source of microbial contaminants in sugars. William L. Owen (*Sugar, N.Y.*, 1955, **50**, No. 8, 44—45, 56).—Thermophilic bacteria are not of common occurrence in raw sugars, but the production of sugar free from mesophilic bacteria is of importance to meat packers. Paper bags used for the distribution of refined granulated sugar may carry the species of acid-producing bacteria responsible for rejection of sugars sold to meat-packers. E. M. J.

Increase in size of saccharide crystals in the presence of non-sugars. Behaviour of single crystals in the presence of colouring matters. L. Cavallaro and G. Mantovani (*Ann. Chim., Roma*, 1955, **45**, 565—574).—The selective adsorption of some colouring substances on particular zones of crystals in process of growth is studied, using an apparatus (illustrated) which allows the growth of a single crystal at a regular rate. Previously-obtained results with cane sugar are repeated, without suggesting that colours are located selectively in particular zones. A relation between chemical structure of the colouring matter and the zone of the sugar is established, in terms of preferential occlusion, in the case of polycrystallisation, rather than selective adsorption. C. A. Finch.

Some physico-chemical methods for the determination of "non-sugars" in the liquors of sugar-mills. L. Cavallaro and A. Indelli (*Ann. Chim. Roma*, 1955, **45**, 539—553).—Present analytical procedures for estimating the quality of the liquors of sugar-mills are discussed, and some physico-chemical methods for the measurement of the "non-sugar" constituents are described. The results of potentiometric titrations are compared with the purity coeff., and a study of instrument errors shows good agreement with the new method. The significance of conductivity measurements is discussed and related to the Stanek–Pavlas method for determination of amine N. A turbidimetric method for the conventional measurement of "colloidal precipitability" is described. C. A. Finch.

Colorimetric method for the determination of invert sugar in the presence of sucrose using 2 : 3 : 5-triphenyltetrazolium chloride. A. Carruthers and A. E. Wootton (*Int. Sugar J.*, 1955, **57**, 193—194).—A sample of test solution containing sucrose and 0·15—0·7 mg. of invert sugar is made up to 4 ml. with distilled water; 1 ml. of N-NaOH and 1 ml. of 1% 2 : 3 : 5-triphenyl-tetrazolium chloride solution are added and the tube is immediately placed in a boiling water bath for 90 sec. It is then immersed in a cold water bath and 1 ml. of 1·1N-acetic acid is added, and within 60 sec. up to nearly 20 ml. of *iso*propanol are added to dissolve all the pptd. formazan. The solution is made up to exactly 20 ml., cooled, and the optical density is measured, using a Hilger Spekker with blue-green filters and 0·25-cm. cells. Invert sugar concentrations can be determined from standard curves drawn up for 0, 0·2, 0·4 g. of sucrose present. Details are given for the preparation of raw juice and carbonatation juices for the test; an excess of Pb and high alkalinity may affect the result. Sug. Ind. Abstr. (E. M. J.).

Glucose as a nutrient, and its status in food legislation. C. Nieman (*Conserva*, 1955, **4**, 42—44).—The physiological and industrial importance of glucose is pointed out, and exception is taken to its legal classification as a substitute for sucrose. P. S. Arup.

Phase equilibria in sugar solutions. X. Quinary system sucrose–glucose–fructose–potassium chloride–water. F. H. C. Kelly (*J. appl. Chem., Lond.*, 1955, **5**, 170—172).—The equilibrium-phase relationships at 30° for this system are shown on a triangular diagram and the influence of KCl under conditions of max concn. are discussed. Saturating the solution with KCl has little effect on the relative proportions of the three sugars present as long as 46% of the sugars is fructose. With decreasing proportion of fructose the proportion of sucrose is markedly reduced (from 65 to 51% in the complete absence of fructose). In general, addition of KCl to an aq. solution of the three sugars increases markedly the solubility coeff. of sucrose, but lowers the proportion of sucrose in the solutes, i.e., reduces the limiting "purity" of the solution with respect to sucrose. J.A.C. Abstr.

Sorbitol: its manufacture and applications. W. H. Erwin (*Chem. Prod.*, 1955, **18**, 205—209).—The production of sorbitol by the continuous catalytic hydrogenation of maize sugar and the subsequent refining of the crude product are described and the properties of sorbitol briefly indicated. Its cosmetic, pharmaceutical, plasticising and food uses are briefly reviewed. J.A.C. Abstr.

Preparation of fresh tomatoes for market. R. L. Spangler and L. E. Ide (*U.S. Dep. Agric.*, 1955, *Fmrs' Bull.* 1291, 45 pp.).—Harvesting, farm and central packing houses, packages, packing, grading and inspection are discussed. E. G. Brickell.

Heterogeneity of carrageenin. David B. Smith, A. N. O'Neill and A. S. Perlin (*Canad. J. Chem.*, 1955, **33**, 1352—1360).—Carrageenin, a polysaccharide complex obtained from red seaweed, can be separated into κ-carrageenin (**I**), pptd. by KCl, and λ-carrageenin (**II**) which remains in solution. Both products are fractionally pptd. with ethanol. The fractions are analysed by paper chromatography, after hydrolysis, and mol. wt. are determined from [η] and sedimentation constants. The carbohydrate residues in **I** are 3 : 6-anhydro-D-galactose (**III**) and D-galactose in nearly equal amounts, with a trace of xylose, probably an impurity. The fractions of **I** are chemically but not physically homogeneous. **I** is not oxidised by periodate. The main fraction of **II** contains only D-galactose and a trace of **III**; materials containing glucose, xylose and L-galactose segregate into minor fractions and hence cannot be integral parts of the principal polysaccharides. **II** is polydisperse on a mass basis and is not oxidisable by periodate. 2% of the rare sugar L-galactose can be readily isolated. (26 references.) A. B. Densham.

Value of dehydrated vegetables as a supplement to the proteins in milled wheat flour and maize meal. B. Sure (*Food Technol.*, 1955, **9**, 413—414).—The supplementary value of several dehydrated vegetables (e.g., lettuce, spinach, cauliflower, etc. used at 5% of rations) to enrich milled wheat flour, and non-enriched maize meal is reported. In rat-growth tests, there were marked increases in growth and pronounced increases in protein efficiency. E. M. J.

Estimation of free starch in potato granules and its relation to consistency of reconstituted product. W. R. Mullins, W. O. Harrington, R. L. Olson, E. R. Wood and Marvel-Dare Nutting (*Food Technol.*, 1955, **9**, 393—395).—A rapid colorimetric method for the estimation of sol. starch is described and its limitations are discussed. E. M. J.

Maturity indices in lima beans. C. Sterling (*Food Technol.*, 1955 **9**, 395—398).—A high rectilinear correlation was found betwee alcohol-insol. solids content in raw and in frozen processed beans ar moisture content, and the ratio: dry wt./fresh vol. (11 reference E. M. J

Quantitative determination of terminal methionine, leucine lysine in raw and toasted soya-bean oil meal. S. W. Fox, C. Wa and T. L. Hurst (*J. agric. Food Chem.*, 1955, **3**, 704—706).—I development of quant. methods of analysis of aminoid and carb terminal amino-acid residues in mixtures of proteins, signi

amounts of methionine are not directly released by the usual heating. Lysine was found to be terminal in both raw and heated protein. The susceptibility of lysine to destruction by heat may result from a relatively exposed position in the protein mol. (24 references.) E. M. J.

Chemistry of citrus fruits. J. F. Kefford (*Rev. pure appl. Chem.*, 1955, **5**, 77—98).—The literature from 1948 to 1954 on the chemistry and technology of citrus products is reviewed. The chemical composition of the fruits is classified on the basis of their general morphology. (197 references.) J. S. C.

Chemical investigation of Indian *Cotoneaster frigida* Wall. I. Chemical composition of fruit pulps. S. K. Datta (*J. Indian chem. Soc.*, 1955, **32**, 344—350).—Data and details of moisture, ash, fat, pentosan, HCN, benzaldehyde, protein, pectic acid and reducing sugars are given. *C. frigida* is particularly rich in D-sorbitol. E. G. Brickell.

Suspected presence of *p*-coumarylquinic acids in tea, apple and pear. R. A. Cartwright, E. A. H. Roberts, A. E. Flood and A. H. Williams (*Chem. & Ind.*, 1955, No. 34, 1062—1063).—Chromatographic techniques have been used to examine tea-leaf, apple and pear leaf and fruit extracts; characteristic, similar patterns were obtained in each case which indicated the presence of a group of *p*-coumarylquinic acids. J. S. C.

Use of fresh fruit objective tests to predict the quality of canned Italian prunes. R. C. Wiley and O. J. Worthington (*Food Technol.*, 1955, **9**, 381—384).—Objective tests on raw prunes, e.g., sol. solids/acid ratio, pressure test, and % of sol. solids, indicate correlation coeff. of 0·90 or higher with the flavour of canned prunes. E. M. J.

Development of a new concept for processing fruit jelly. W. J. Hoover, A. I. Nelson and M. P. Steinberg (*Food Technol.*, 1955, **9**, 377—380).—Divided into three portions the fruit juice (apple) was treated as follows: (i) was heated up to 180°F. to dissolve the sugar and pectin; (ii) was boiled to evaporate the required amount of water; (iii) was not heated until it was mixed with the other two portions and the whole was heated to 180°F. before filling the jars, to give a jelly of definitely superior quality to that produced by a simulated commercial process. E. M. J.

Utilisation of fruits in food products. W. V. Cruess (*Food Technol.* 1955, **9**, 419—425).—The report of the Babcock–Hart Award address 1955. (58 references.) E. M. J.

Reconstituting moisture in over-dried popcorn by blending with wet popcorn. W. A. Huelsen and W. P. Bemis (*Food Technol.*, 1955, **9**, 426—430).—Blending of over-dried popcorn with wet popcorn in the proportions to bring the blend to 12·5% theoretical moisture is recommended as one commercial method of reconstitution. E. M. J.

Natural inhibitors against ascorbic acid oxidase contained in egg apples. C. Inagaki, H. Fukuba and A. Matsushita (*Nat. Sci. Rep. Ochanomizu Univ.*, 1955, **5**, 313—322).—Two inhibitors of ascorbic acid oxidase were found in egg-apple juice: (1) a thermostable anthocyanin pigment, nasunin, mainly contained in the pericarp, and (2) a thermolabile substance, mainly contained in the sarcocarp, which had the characteristics of a globulin. Since the latter inhibits ascorbic acid oxidation with Cu sulphate, it is suggested that its inhibitory action may be derived from combination with the prosthetic group of the oxidase. J. S. C.

Distribution of the natural inhibitors against cucumber ascorbic acid oxidase. C. Inagaki, H. Fukuba and A. Matsushita (*Nat. Sci. Rep. Ochanomizu Univ.*, 1955, **5**, 323—331).—The inhibition of the ascorbic acid oxidase of cucumber was measured with 47 kinds of vegetable and fruit juices. A 100% inhibition was obtained with juices from pumpkin, burdock, spikenard, shungiku (*Chrysanthemum coronarium*), lettuce and egg-apple. Juices from peach, water melon (sancocarp), radish, cabbage, apple, white musk melon (*Cucumis conomen*) and sweet pepper showed no inhibition. It is assumed that the inhibitory action arises from the combination of the prosthetic groups of an ascorbic acid oxidase and another type of oxidase, forming a Cu–Cu linkage. J. S. C.

Preparation of ascorbinase from melon (*Citrullus vulgaris*). L. V. le Farias and B. M. Neto (*Rev. Quím. industr., Rio de Janeiro*, 1955, **4**, 25).—Ascorbinase is a conjugated protein containing some Cu id is met in various vegetables, particularly in the *Cucurbitaceæ*. was isolated from *C. vulgaris* by crushing the fruit and passing the ste through a sieve. The liquid obtained was more or less ured, depending on the part of the fruit used, and was treated a solution of Ba acetate corresponding to 4% of the vol. . The supernatant liquor was treated with 10% by vol. of ted NH_4 sulphate solution, and allowed to stand in a separat- nnel. The flocculent portion, containing the enzyme, was dialysed through a Cellophane membrane for 24 hr. in running water, leaving the enzyme behind. H. Pritchard.

Use of silver preparations for sterilising sugar-containing liquids. U. Kutscher (*Mschr. Brauerei wissen Beil.*, 1955, **8**, No. 8, 95—97).—Five different Ag prep. were tested against various species of bacteria, yeasts and mucor using five different substrates. Most of the tested organisms were neither inhibited nor killed. The application of such Ag processes to the conservation or sterilisation of alcohol-free beverages and other solutions is found to be unreliable and biologically doubtful. E. M. J.

"Œnometrie": some complementary data. L. Deibner (*Rev. Ferment. Industr. aliment.*, 1955, **10**, 125—131; cf. J.S.F.A. Abstr., 1955, i, 125, 259).—The earlier reviews of quant. data relating to the properties and manufacture of wines are supplemented by studies of sp. heat and thermal conductivity, CO_2 pressure and absorbency-coeff. of sparkling wines, control of secondary fermentation, froth-forming characteristics, the use of statistical methods, and stability forecasting (tartrate deposition). (23 references.) J. S. C.

Laboratory wine-press with filter for extraction of juice from grapes in inert atmosphere and with variable, known pressures. L. Deibner (*Industr. agric. aliment.*, 1955, **72**, 323—328, 407—411).—Pressure is applied to grapes contained in a stainless steel cylinder by means of a piston actuated by a lever, at the end of which known weights are applied. The juice is filtered through a Buchner funnel and discharged into a vessel fitted with electrodes for potentiometric measurement of oxidation–reduction potential. A stream of N_2 is passed through the apparatus so that at no point does the juice come into contact with air. J. S. C.

Microbiological assay of *meso*inositol using *Klœckera apiculata* and *Saccharomyces veronæ*: application to grapes and wines. J. Ribereau-Gayon, E. Peynaud and S. Lafourcade (*Rev. Ferment. Industr. aliment.*, 1955, **10**, 119—121).—Four series of tubes, each containing 10 ml. of culture medium (composition stated), with added amounts of *meso*-inositol (**I**) varying from 0·25—2·5 μg., were each inoculated with one of each of the following: *K. apiculata* (**II**), *Sacch. veronæ* (**III**) and *Torulopsis bacillaris* 46 and 61. Each tube was incubated for 40 hr. at 25° and the optical density then determined with an absorptiometer. The relation between **I** content and optical density was found to be approx. linear with **II** and **III**. The technique was applied to the assay of **I** in wines and in grape juices during the ripening period. Wine should be de-sulphited, diluted (1 : 1000) with distilled water, and samples of 1, 2, 3 and 4 ml. used for the assay as previously described. Alternatively, the wine may be clarified with neutral Pb acetate and **I** pptd. with basic Pb acetate in ammoniacal solution, redissolving the ppt. in aq. H_2SO_4, separating from $PbSO_4$ by filtration and diluting the filtrate and washings to a suitable aliquot. (14 references.) J. S. C.

Skin pigments of the Cabernet Sauvignon grape and related progeny. A. H. Bockian, R. E. Kepner and A. Dinsmoor Webb (*J. agric. Food Chem.*, 1955, **3**, 695—699).—Skin pigments of the Cabernet Sauvignon grape were separated by chromatography and identified as malvidin, two glucosides of malvidin, a petunidin glucoside, a delphinidin glucoside and a complex diglucoside of malvidin which was present in greatest quantity. The pigments of progeny of Cabernet Sauvignon crossed with Carignane grapes were qualitatively identical with those of Cabernet Sauvignon. The first anthocyanin pigment to appear was the malvidin diglucoside, then malvidin monoglucoside and delphinidin glucoside, then petunidin glucoside. Free malvidin was observed one week after the berries had attained complete maturity. In *Coreopsis* the anthocyanins developed at the expense of the corresponding anthocyanidins. (20 references.) E. M. J.

Improvement of quality of wine or sweet musts by refrigeration. III. W. Saller (*Mitt. Wein- u. Obstbau, Wien*, 1955, **5, A**, 229—262; cf. *ibid.*, 101, 157).—Suitable types of cooling plant and methods of operation are described for low-temp. pressure-tank fermentation, for the cooling of wines in order to accelerate the deposition of tartar, and for supersaturating wines or sweet musts with CO_2. The author's automatic pressure-tank fermentation regulator actuates the cooling system when the pressure in the tank reaches a certain limit, and *vice versa*. The advantages of controlled over ordinary fermentation, as regards the composition and quality of the product, etc., are comprehensively reviewed. (50 references.) P. S. Arup.

Retardation of fermentation [of wine]. H. Konlechner and H. Haushofer (*Mitt. Wein- u. Obstbau, Wien*, 1955, **5, A**, 263—271).—Retardation by filtering or centrifuging off part of the yeast during fermentation gives greater improvement in the quality of the wine than does controlled low-temp. pressure-tank fermentation. Clarification of the must by settling retards fermentation with good

results; retardation by means of SO_2 gives poor results. (15 references.) P. S. ARUP.

Cloudiness in wines. W. Draper and J. L. Thomson (*Chem. Can.*, 1955, **7**, No. 8, 35—38).—The merits and techniques of the blue fining process using K ferrocyanide for removing turbidity in wine caused by presence of Cu and Fe are reviewed. D. R. PECK.

Comparative study of the alterations in the organoleptic properties of wine produced by the addition of certain aliphatic acids with special reference to butyric acid which is generally considered to be the cause of the butyric acid sourness of wine. H. Mohorčič (*Vestnik Slovensk. kemijsk. Društ.*, 1955, **2**, 1—7).—Traces of aliphatic acids added to wine affect the taste and smell. Butyric acid is detected in wines showing butyric acid sourness, confirming that the acid is the cause of such sourness. It is disguised by treatment with SO_2, but reappears when the excess of SO_2 disappears. These effects, and the action of propionic acid in paralysing the ferment, are probably related to the pH of the wine. A. B. DENSHAM.

Determination of free and total sulphur dioxide in white table wines. M. A. Joslyn (*J. agric. Food Chem.*, 1955, **3**, 686—695).—Two methods for the determination of free and total SO_2 by iodimetric titration and colorimetric fuchsin–formaldehyde were critically examined for factors affecting accuracy and reproducibility of the results. In the first, e.g., the concentration of the starch affected the sharpness of the end-point, and the speed of titration affected the reproducibility of the results. The concentration of H_2SO_4 in the acid-bleached fuchsin reagent had the greatest effect on sensitivity. (33 references.) E. M. J.

Use of sodium hexametaphosphate for preventing formation of crystalline deposits in bottled wines. F. Epp (*Mitt. Wein- u. Obstbau, Wien*, 1955, **5, A**, 276—280).—Trials with this salt gave unsatisfactory results for early bottled wines with moderately high org. acid content, stored at −4°. P. S. ARUP.

Acrolein in spirits. L. Rosenthaler and G. Vegezzi (*Z. Lebensmitt-Untersuch.*, 1955, **102**, 117—123).—Appreciable amounts (0·0001—0·02% v/v) of acrolein (**I**) were detected by means of the author's reaction (cf. *ibid.*, 1955, **101**, 33) in a large no. of brandies of diverse origins, but not in genuine brandies of commerce. Contents of **I** in brandy or pure aq. EtOH are considerably reduced, but do not entirely disappear after storage during 6 months. Brandy containing 0·01% (v/v) of **I** was consistently freed from it in a continuous rectifier on a factory scale, the **I** being confined to the first and to the fusel-oil fractions. Laboratory experiments on the production of EtOH from glucose, under aseptic and ordinary conditions, indicated that the formation of **I** may be due to infecting microorganisms. P. S. ARUP.

History of technical microbiology in U.S.S.R. II. Spirits industry. I. L. Rabotnowa (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 102—105).—A review with 73 references. P. S. ARUP.

Theory and practice of alcohol determination. III. K. Rokitansky (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 37—43).—An explanation is given of the calculations used in constructing the diagram previously given (cf. J.S.F.A. Abstr., 1955, ii, 32) for ascertaining, in terms of the original vol. of the still-liquid and its % of EtOH, the vol. of liquid to be distilled off in order to reduce the % of EtOH of the residual liquid to a given value. P. S. ARUP.

Reliability of prediction tests for malting quality of barley. V. M. Bendelow and W. O. S. Meredith (*Canad. J. agric. Sci.*, 1955, **35**, 252—258).—Prediction tests carried out on 243 hydrid lines by standard methods used in Canada proved successful for 79% of the lines subsequently found to have good malting quality. The non-inclusion of the other good lines was due to the underestimation of saccharifying power which occurs with hybrids of Peatland parentage. P. S. ARUP.

Contributions to colour and turbidity measurement in wort and beer with a new small Zeiss electrophotometer. H. Hecht (*Brauwissenschaft*, 1955, 175—178).—A new Zeiss electrophotometer Elko II is described and illustrated which is said to eliminate errors associated with personal observation of colour in beer and wort. E. M. J.

Amounts of pteroylglutamic acid and related compounds in different yeasts. V. M. Doctor and W. H. Peterson (*Appl. Microbiol.*, 1955, **3**, 29—34).—The apparent pteroylglutamic acid (**I**) content of 18 yeasts is determined. Of the total **I** produced by the yeasts <10% passed into the medium. Of that retained in the cells 70—90% was in a bound form from which it was released by suitable enzymes, notably those of chick pancreas or hog kidney. Production of **I** by *Saccharomyces* exceeded that by *Torulopsis* or *Candida* types. The proportions of **I** and its formyl derivative (folinic acid) in experimental and commercial yeasts grown on various media are recorded: the **I** contents ranged from 31—65% of the total activity in experimental, and from 17—82% in commercial yeasts. A. G. POLLARD.

Antibiotic properties of carbohydrate-fermenting yeasts. A. M. Skorodumowa (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 43—46).—A no. of yeasts produce inhibition-zones on agar media with respect (especially) to Gram-positive bacteria (including several common pathogenic types), provided that a carbohydrate is present which can be fermented by the yeast under observation. The (crude) antibiotic (Saccharamyzetin) is heat-stable, gives no biuret reaction, and is inhibited by the presence of peptone in the medium; its production is closely connected with the fermentation process. P. S. ARUP.

Death rate of yeasts under toxic effects in relation to the number of chromosomes. F. Weinfurtner and G. A. Voerkelius (*Brauwissenschaft*, 1955, 178—179).—The effects of toxic substances such as H_2SO_4, $HgCl_2$, quaternary NH_4 compounds, etc., and of u.v. and γ-rays on the production of mutations in genes and chromosomes are discussed. Death rate curves of haploid, diploid and tetraploid yeasts are given. E. M. J.

Foam stability of beer. IV. Effects of boiling the wort and addition of hops. W. Piratzky, H. Beitner, J. Jacker and B. Nispel (*Brauwissenschaft*, 1955, 172—174; cf. J.S.F.A. Abstr., 1955, ii, 183, 244).—A critical study was made on the effect of boiling under reflux and in an autoclave, without the addition of hops, on the foam stability of wort. Heating caused considerable variation in foam production, and the unfavourable effect was greater on heating >100°. Of the resin constituents of hops, lupulone improved the foam formation most; tannin had only an insignificant effect and that of the N-containing substances was negligible. E. M. J.

Carbonation [of beverages]. Physico-chemical aspects. R. Steenhoff (*Svensk. Bryggeritidskr.*, 1955, **70**, 167—179).—A review. P. S. ARUP.

Identification of ascorbic acid in maté infusions. R. Ramos Barreto (*Rev. Quím. industr., Rio de Janeiro*, 1955, **24**, 17—18).—Full details are given of a procedure for the identification of ascorbic acid by a chromatographic method, using a control amount of the acid. It was established that infusions of maté (*Ilex paraguayensis*) contain ascorbic acid, at least in its oxidised form. H. PRITCHARD.

Spectrophotometric determination of total methylxanthine [alkaloids] in refreshing beverages containing caffeine. A. Schaller and H. Klaushofer (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 105—112).—A review is given of published data on the composition of the above beverages, the alkaloid content of the ingredients cola-nut and maté-tea (chiefly caffeine and theobromine), and the spectrophotometric determination of these alkaloids. The sp. extinction values ($E^{0\cdot001\%}_{1\,cm.}$) of caffeine and theobromine are determined at 272 mμ. as 0·05081 and 0·05512, respectively, and a method for their joint determination (as caffeine) is described: an aq. solution of the alkaloids for spectrophotometric measurement is prepared by first clearing the sample by treatment with aq. $CuSO_4$, NaOH and $Pb(OAc)_2$, then extracting the alkaloids from the acidified solution by means of $CHCl_3$, and finally evaporating the $CHCl_3$ extracts and dissolving the residue in water. The method is accurate within ±1%. Preliminary experiments on the determination of the total methylxanthine alkaloids in maté-tea are described. (42 references.) P. S. ARUP.

Costs: process and construction. W. L. Hardy (*Industr. Engng Chem.*, 1955, **47**, No. 8, 79A—81A).—The process for producing soluble or "instant" coffee and equipment used are outlined, and costs are given and discussed. O. M. WHITTON.

Preservation of milk by heating. T. E. Galesloot (*Conserva*, 1955, **4**, 48).—The bacteriological shortcomings of the usual methods of pasteurisation are pointed out; ideal conditions would consist in high-temp. short-time sterilisation followed by bottling in sterile bottles. P. S. ARUP.

Determination of the colour of milk and milk products. F. H. Tinkler, R. C. Stibley and F. W. Bernhart (*J. Dairy Sci.*, 1955, **38**, 634—639).—Modifications are described for improving the method of Choi *et al.* (*ibid.*, 1949, **32**, 580) for the determination of the browning of milk products. The natural and heat-produced colours c skim and whole milk and of some milk powders are reported. Cu rent sterilisation processes cause greater browning than do dryi techniques. S. C. JOLLY.

Effect on flavour of using substitute fats in dry whole milk. C Herald and S. Patton (*J. Dairy Sci.*, 1955, **38**, 640—644).—W milk powder having a good flavour and satisfactory keeping qua can be prepared by replacing the milk fat by either hydrogena unhydrogenated coconut oil. Partially hydrogenated cotto

groundnut and soya-bean oils and lard were unsatisfactory as substitutes. S. C. JOLLY.

Device for collecting and rapidly cooling samples from high-temperature short-time heating units. O. W. Kaufmann, J. Tobias and H. Wainess (*J. Dairy Sci.*, 1955 **38,** 645—650).—A device is described for collecting and cooling samples from high-temp. short-time units for bacteriological examination. A method is given by which the adequacy of a cooling process may be evaluated. S. C. JOLLY.

Slow acid production by lactic cultures. F. J. Babel (*J. Dairy Sci.*, 1955, **38,** 705—733).—Factors that influence acid production by lactic cultures and methods of reducing the incidence of slow acid production are reviewed. (146 references.) S. C. JOLLY.

Electrochemical behaviour of ion-exchange-resin-membrane electrodes. H. E. Affsprung, C. W. Gehrke and J. W. Browne (*J. Dairy Sci.*, 1955, **38,** 734—742).—The prep. of membranes from various cationic and anionic exchange materials and their electrochemical behaviour are described. Cationic exchangers used were Amberlite IR-120, Amberlite IRC-50, Decalso, Dowex-50, Duolite CS-100, Permutite H and Permutite Q. Anionic exchangers used were Amberlite IRA-400 and Dowex-2. S. C. JOLLY.

Influence of certain factors on the bacterial counts and proteolytic activities of several psychrophilic organisms. W. C. van der Zant and A. V. Moore (*J. Dairy Sci.*, 1955, **38,** 743—750).—Incubation for three days at 25° is recommended for the enumeration of bacteria growing in refrigerated milk and related products. Three of the four cultures tested grew over a wide range of temp. and had a lag phase of >24 hr. There was no correlation between proteolytic activity and population. There was apparently some relation between the production of sol. N and tyrosine and tryptophan. S. C. JOLLY.

Extraction–titration method for the determination of free fatty acids in rancid milk and cream. E. N. Frankel and N. P. Trassuk (*J. Dairy Sci.* 1955, **38,** 751—763).—A simple and direct extraction–titration method is described for the determination of free fatty acids in rancid milk and cream. Recoveries of 95—100% are obtained for added high-mol.-wt. and 52—58% for added low-mol.wt. fatty acids. Approx. 90% of the fatty acids formed by hydrolysis of milk fat can be estimated by this method, compared with approx. 65% by the churned-fat method. Previous lipase studies based on the acid degree of churned fat are valid, however, when the value was used for determining only the relative extent of rancidity. S. C. JOLLY.

Photometric method for estimating the lipase content of milk. T. L. Forster, C. Jensen and E. Plath (*J. Dairy Sci.*, 1955, **38,** 764—774).—A photometric method is described for estimating the lipase content of whole milk using the butyryl, hexanoyl and octanoyl esters of 2-hydroxynaphthalene-6-sulphonic acid as substrates. The synthesis and properties of these esters are described. S. C. JOLLY.

Selective release of volatile acids from butterfat by microbial lipases. J. C. Wilcox, W. O. Nelson and W. A. Wood (*J. Dairy Sci.*, 1955, **38,** 775—781).—Lipases from 2 of 9 cultures of *Goetrichum candidum* hydrolysed only butyric acid (**I**) from butterfat; the other 7 cultures produced no volatile fatty acids. Lipases of *Candida lipolytica* produced **I**, and some also produced hexanoic acid (**II**). **I**, **II** and octanoic acid (**III**) were released by lipases of *Penicillium roqueforti* and *Achromobacter lipolyticum*. Lipases of *P. camemberti* released **I** and an unidentified volatile acid which had a R_F value between those of **II** and **III**. S. C. JOLLY.

Detection and quantitative determination of colouring materials in butter and margarine. H. S. Espoy and H. S. Barnett (*Food Technol.*, 1955, **9,** 367—372).—Tests were made with melted butter or margarine samples from a solution of 30—35 g. of weighed oil made up to 100 ml. in hexane. The colouring matter, carotene, coal tar colours, or annatto was identified and measured spectrophotometrically, without saponifying the oil. (15 references.) E. M. J.

Adjustment of casein–fat ratio of milk for cheesemaking. R. M. Dolby and W. L. Harkness (*N.Z. J. Sci. Tech.*, 1955, **A, 37,** 68—82).—A rapid method for standardising milk for cheesemaking is described; the milk is sampled in the receiving vat while supplies re arriving. Its accuracy has been confirmed in factory trials. he relationship between casein/fat ratio in milk and fat in the ıter-free solids in cheese is discussed. N. M. WALLER.

Biochemistry of cheese ripening. XIV. Occurrence of lysine ırboxylase in ripening sour milk cheese. J. Schormüller and Leichter (*Z. LebensmittUntersuch.*, 1955, **102,** 97—106; cf. ˙.A. Abstr., 1955, ii, 246).—A previously-described technique ·id., 1953, **96,** 1; **97,** 446) is used for estimating the activity enzyme in suspensions of the cheese with added lysine. The pH optimum is 5·1—5·8. Pyridoxal-5′-phosphate stimulates this enzyme to a marked degree, and restores its lost activity after removal of water-sol. constituents, or after ageing of the cheese during 10 days. Hydroxylamine and semicarbazide in low concn. inhibit the enzyme completely. The significance of these findings is considered. Cadaverine is identified by means of paper chromatography as a product of the decarboxylation of lysine. (53 references.) P. S. ARUP.

Pasteurisation of commercial eggs. P. S. Watts and E. L. Vawser (*Aust. J. appl. Sci.*, 1955, **6,** 255—260).—The keeping properties and quality of slightly-dirty or dirty eggs from collecting centres can be greatly improved by immersion in water at 63° for 2—3 min. immediately after mechanical washing in cold water on a tape machine. Operating details of this laboratory "pasteurising" plant are given. The treated eggs can then be stored in the cold for up to 90 days followed by keeping at room temp. for 10 or 20 days. Without the heat treatment ~60% of the eggs would become unusable after 3 months' storage. The % rots in treated eggs after 30 days' cold-storage and 20 days' keeping at room temp. was <2 compared with 7·5 for eggs washed but not heat-treated. Bacteria isolated from rotted, treated eggs were resistant up to 65° for $\not<10$ min., so that no practicable heat-treatment would have killed them. W. J. BAKER.

Gossypol–cephalin compound from fresh eggs of hens fed cotton-seed meal. C. L. Woronick and C. R. Grau (*J. agric. Food Chem.*, 1955, **3,** 706—707).—Egg yolks from hens fed gossypol contain a distinctive yellow component having a direct relationship with the amount of gossypol fed. The absorption spectra of gossypol egg cephalin has peaks at 380 and 400 mμ. Gossypol was identified in the cephalin fraction after oxalic acid hydrolysis. As gossypol readily reacts with amino-groups, it is concluded that the primary amino-groups of egg cephalin condense with the aldehyde groups of gossypol to form a Schiff base. (12 references.) E. M. J.

Effect of salt content on microbial spoilage of edible egg-yolk. K. Raible (*Zbl. Bakt.*, 1955, II, **108,** 588—602).—Microflora obtaining predominance at 25° in unsalted and salted egg-yolk, previously infected with eight spoilage organisms, are: in unsalted, Gram-negative rods, especially *Bacillus cereus* and streptococci; with 3—4% NaCl, Gram-negative rods, streptococci and micrococci; with 5—8% NaCl, proteolytic micrococci, which can include *Micrococcus pyogenes*. The cheesy odour and tendency to coagulate observed for unsalted yolk disappear with increasing NaCl contents. Changes in pH are examined. Storage in an atm. free from O_2 does not affect bacterial growth. P. S. ARUP.

Changes in the oxidation–reduction potential of *sterno-cephalicus* muscle of the horse after death in relation to the development of bacteria. E. M. Barnes and M. Ingram (*J. Sci. Food Agric.*, 1955, **6,** 448—455).—Changes in the oxidation–reduction potential of intact horse muscle, kept under anaërobic conditions were studied for 1—2 days *post mortem*, and *rigor mortis* changes were followed concurrently by means of pH determinations. The change in potential, i.e., from $E_H > +250$ to $\sim E_H -130$ mv. occurred mainly within the first 3—4 hr. at 37°, and the end of the rapid fall appeared to coincide with the onset of *rigor*. After *rigor mortis* the potential remains steady if bacterial growth is inhibited, either by low temp. conditions or treating the muscle sample with aureomycin (1—2 p.p.m.). The low potential values obtained under normal conditions ~−250 mv. result from the metabolic activity of bacteria. E. M. J.

Effect of different methods of cooking beef round of commercial and prime grades. I. Palatability and shear values. II. Collagen, fat, and nitrogen content. R. M. Griswold (*Food Res.*, 1955, **20,** 160—170).—I. Cooking data, palatability scores, and shear values for meat of animals of commercial and prime grades are presented. Because of variations in the quality of meat from the rump to the shank end of beef round, the cooking methods are arranged in groups, each containing a standard braising method. Direct comparisons are made within one group. Comparisons of the difference of a method and its standard may be made between groups. Standard braising was preferred to braising under pressure. Pounding but not scoring increased tenderness. Beef round roasted at 250°F. scored high in flavour of lean and acceptability, and had lower shear values than meat cooked by many other methods. Meat roasted at 300°F. was superior only in juiciness to that roasted at 250°F. Palatability scores were on the whole higher and shear values were lower for beef of prime than of commercial grade.

II. Analytical data are presented on 15 methods including a standard braising method of cooking beef round of commercial and prime grades. The collagen content, in raw and cooked meat, moisture, fat, pH, total N, and free amino-N content of the meat and drippings were determined. The more soluble nitrogenous constituents went into the drippings, and the total amount of free amino-N did

not increase during cooking. Cooked beef of commercial grade contained significantly (1% level) more collagen than cooked meat of prime grade. Collagen losses on cooking by all methods averaged 61%; losses increased as the internal temp. of the meat increased; or with increase of pressure in cooking; they were greater in meat roasted at 250°F. than at 300°F., or braised by the standard method, and were great in meat soaked in vinegar. Collagen loss in meat braised without water was less than for any other method. E. M. J.

Dehydrated pork studies. Removal of glucose by yeast fermentation. R. L. Henrickson, D. E. Brady, C. W. Gehrke and R. F. Brooks (*Food Technol.*, 1955, **9**, 290—292).—Lean meat (95%) from fresh ham of $\frac{1}{2}$-, $\frac{1}{4}$-, $\frac{1}{8}$-in. particle size was used. Washed yeast cells (*Saccharomyces cerevisiæ*) were used to remove the glucose by fermentation, approximately 50% being removed from pork of particle-size $\frac{1}{8}$ in. This grind of meat was the most practical as judged by the taste panel evaluation for colour, texture, and flavour of the stored meat. Pork fermented with 5 and with 10% yeast for a period of four hours gave the most desirable dehydrated product. Off flavours, caused by high bacterial populations in raw pork, were minimised by cooking the pork before fermentation. If this meat was then dehydrated the shelf life of the dehydrated product was further improved. E. M. J.

Variation of ultimate pH within pig muscles. J. F. Scaife (*J. Sci. Food Agric.*, 1955, **6**, 467—471).—Wide variations of ultimate pH were found within single muscles in pigs, the variations being correlated with the distribution of myoglobin. There was an uneven distribution of pigment also, and a highly significant correlation was found between pH and pigment concentration. Relevant literature is discussed. (22 references.) E. M. J.

Freeze-dried meat. I. Preparation and properties. A. L. Tappel, A. Conroy, M. R. Emerson, L. W. Regier and G. F. Stewart (*Food Technol.*, 1955, **9**, 401—405).—The freeze-drying and resulting properties of pieces 1 in. thick, of beef *biceps femoris* and various serving-size pieces of meat, fish and poultry are described. (13 references.) E. M. J.

Effect of fluctuating temperatures on frozen turkeys. A. A. Klose, M. F. Pool and H. Lineweaver (*Food Technol.*, 1955, **9**, 372—376). E. M. J.

Yeasts from commercial meat brines. R. N. Costilow, J. L. Etchells and T. N. Blumer (*Appl. Microbiol.*, 1954, **2**, 300—302).—Of 89 yeast isolates from casks of commercially brined meat (hams, beef tongues, bacon sides, and Canadian bacon) 86 were identified as *Debaryomyces membranæfaciens* var. *Hollandicus* Lodder and were responsible for film formation on the brines. The remaining three, which were sub-surface and non-film-forming, were placed as *D. klockeri* Guill. et Péju. E. G. BRICKELL.

Gamma-ray sterilisation of canned meat previously inoculated with anaërobic bacterial spores. L. L. Kempe, J. T. Graikoski and R. A. Gillies (*Appl. Microbiol.*, 1954, **2**, 330—332).—The amount of γ-radiation required to sterilise meat in cans varied linearly with the concn. of spores of *Clostridium botulinum*, e.g. 2,5000,000—4,000,000 rep from ^{60}Co radiation for spore loads of 0·4—40,000 per g. of meat. A. G. POLLARD.

Ices containing chlorotetracycline in experimental fish preservation. D. C. Gillespie, J. W. Boyd, H. M. Bissett and H. L. A. Tarr (*Food Technol.*, 1955, **9**, 296—300).—Methods of preparing flake-type, and block ice in which chlorotetracycline (CTC) is uniformly distributed are described. Blocks of ice were prepared by the normal commercial slow-freezing procedures; the "distributing agents" used were, *e.g.*, Hercules carboxymethylcellulose 70, high viscosity; carrageen; an alginate ("Dricoid"); or "Difco" gelatin; etc. When these ice preparations containing about 1 p.p.m. of CTC were applied to eviscerated fish on fishing boats, bacterial spoilage was markedly delayed. The inclusion of 1 to 4 p.p.m. of CTC in refrigerated sea water at −2° greatly improved the keeping quality of both eviscerated and fish which were not eviscerated for nine days, when transported in this medium. Experiments carried out at sea and ashore indicate that a long interval *post mortem*, before antibiotic ice is applied, too short an application, or poor icing procedures all interfere with its effectiveness. (12 references.) E. M. J.

Inhibition of mould and yeast development in fish products. J. W. Boyd and H. L. A. Tarr (*Food Technol.*, 1955, **9**, 411—412; cf. *Fish Res. Bd Canada Prog. Rep. Pacific*, 1954, **99**, 22).—Growth of moulds, e.g., *Sporendonema epizoum* and yeasts in smoked fish was strongly delayed when 0·05—0·1% of sorbic acid was incorporated in the flesh during the brining process. E. M. J.

Detection of foreign animal fats in chicken-fat. C. Franzke (*Z. LebensmittUntersuch.*, 1955, **102**, 81—84).—The fats are examined for the detection of beef or pork in chicken meat in cases where histological or serological examination is inapplicable. Differences between the constants for beef and lard on the one hand, and chicken-fat on the other, are in the following (steeply ascending) order for: I val., yield of polybromides from the unsaturated fatty acids, and spectrophotometric extinction val. determined at 233 mμ. on the alkali-isomerised fatty acids. P. S. ARUP.

Liver oil of a shark (*Centrophorus granulosus*). I. Separation of squalene from the unsaponifiable matter. F. Ramos Ayerbe and M. A. Albi Romero (*Grasas y Aceites*, 1955, **6**, 141—143).—The squalene can be separated from the unsaponifiable fraction of the oil by adsorption on an alumina column, or by distillation under reduced pressure. L. A. O'NEILL.

Determination of vitamin A in natural products and especially cod-liver oils. R. A. Morton and F. Bro-Rasmussen (*Analyst*, 1955, **80**, 410—418).—The analytical procedures of the B.P. and U.S.P. for the determination of vitamin A are discussed and the newer problems arising out of the presence of three vitamin-A-active substances, viz., all-*trans*-vitamin A_1, neovitamin A_1 and vitamin A_2, in fish-liver oils. While very suitable for products containing synthetic all-*trans*-vitamin A, these procedures are less precise for those containing mammalian liver oils and concentrates. Their application to the determination of vitamin A in cod-liver oil, for example, is an officially sanctioned convention giving a serviceable approximation; the nature of this is discussed. Application of the Morton–Stubbs correction to the absorption graph of a natural oil tends to eliminate the vitamin A_2 and to over-correct for the neovitamin A_1. This over-correction is balanced by the lower potency of the neovitamin, so that the B.P. and U.S.P. procedures yield a fair estimate of the vitamin-A_1 potency. The properties, biological potency and absorption spectra of the three active substances are discussed with special reference to conversion factors. A. O. JONES.

Chromatographic separation of vitamin-A-active compounds in cod-liver oil. F. Bro-Rasmussen, W. Hjarde and O. Porotnikoff (*Analyst*, 1955, **80**, 418—428).—A method is given for the chromatographic separation of neovitamin A_1, all-*trans*-vitamin A_1 and vitamin A_2 on activated dicalcium phosphate columns 300 to 600 mm. long, the eluent being a mixture of light petroleum and peroxide-free ether. Absorption curves are given for the three active substances. Fractionation graphs (extinction at 325 mμ. against vol. of eluate) show an efficient separation of the three components from unsaponifiable fractions of cod-liver oil. Good agreement is found between the determined content of the three compounds and the total vitamin A found by the normal chromatographic procedure. A. O. JONES.

Modified method for the spectrophotometric determination of vitamin A in margarine. J. W. Lord and F. M. Bradley (*Analyst*, 1955, **80**, 429—438).—A method is described for the determination of vitamin A in margarine similar in principle to the official method (Statutory Instrument, 1954, No. 613) but with de-fatted bone meal as the adsorbent. The unsaponifiable fraction of the margarine is applied to the column in light petroleum and the column is washed with this solvent until no more carotene or dye appears in the eluate. It is then eluted with a mixture of light petroleum and peroxide-free ether, the eluate being collected in 5-ml. fractions. The fractions containing vitamin A are identified by the Carr–Price test and aliquot portions are combined and adjusted to a definite vol. From the optical density at 325 mμ. (subject to a correction) the vitamin-A potency of the margarine is calculated. For practical purposes the results are in good agreement with those of the official method. The method described has the advantage of using a commercially-available adsorbent that is more stable than the alkaline alumina of the official method. A. O. JONES.

Rapid method for determination of the oil-content of the outer pulp and skin of olives. C. Brés and L. Vincent (*Oléagineux*, 1955, **10**, 559—564).—The method described is based on refluxing the sample with trichloroethylene (**I**) and obtaining the sp. gr. of **I** before and after doing so. The difference is used to calculate the oil content of the sample. Nomograms are given to facilitate calculation and correction for temp. J. S. C.

Seed oils of *Bombax sessile* and *Lupinus termis*. D. N. Grindle A. A. Akour (*J. Sci. Food Agric.*, 1955, **6**, 461—465).—The se of *Bombax sessile* and of *Lupinus termis* contain respectively ides of palmitic, oleic and linoleic acids; and of oleic, saturated fatty acids of high mol. wt. e.g., palmitic, ar amounts of linolenic acid. The composition of the seed species and the detailed composition and properties of th are given.

Chromatographic study of the action of pancreatic lipa triglycerides. P. Savary and P. Desnuelle (*C. R. Acad* 1955, **240**, 2571—2573).—The chromatographic techniq described (cf. J.S.F.A. Abstr., 1955, i, 136) was used

products of lipolysis of a mixture of palmitodiolein (dissymmetric) and oleopalmitin, both symmetric and dissymmetric, treated with pancreatic lipase. The products contained, in order of predominance, 1 : 2-diglycerides, 2-monoglycerides and glycerol. The chains in positions 1 and 3 were most readily detachable. It is suggested that the 1-oleic chains are more quickly detachable than the 1-palmitic chains but that this specific action is less marked than the effect of position in the structures of the triglycerides. J. S. C.

Stabilisation of autoxidisable materials by means of inclusion. H. Schlenk, D. M. Sand and J. A. Tillotson (*J. Amer. chem. Soc.*, 1955, **77**, 3587—3590).—Adducts of α- and β-dextrin and deoxycholic acid with linoleic acid, linolenic acid, methyl linolenate, cinnamaldehyde and vitamin A palmitate were found to be very resistant to autoxidation. These examples confirm previous work on the principle of inclusion stabilisation. (20 references.) J. S. C.

Solvent extraction of cottonseed. W. G. Juhl (*Iowa St. Coll. J. Sci.*, 1955, **29**, 441—442).—The extraction by trichloroethylene of cottonseed oil from flaked cottonseed meats was studied in a pilot plant consisting of a counter-current, continuous unit. The operating variables considered were: extraction time, meat diameter, flake thickness, extraction temp., oil, concn. in solvent of miscella, moisture content of the flakes, and degree of heating the meats. Mathematical relations were developed expressing the individual effects of the first four variables on % of extractable material remaining in the processed flakes. An equation was developed relating the residual extractables to kinematic viscosity and extraction temp., and a further equation relating these variables also with flake thickness, meat diameter and extraction time deduced. J.A.C. Abstr.

General density equation for glyceride oil–solvent mixtures. Calculation of density–composition–temperature data. E. L. Skau, F. C. Magne, R. R. Mold and R. L. Durr (*Industr. Engng Chem.*, 1955, **47**, 1043—1049).—A simple quadratic equation for calculating directly the *d* or the composition of any glyceridic oil–solvent mixture from the *d* of the oil and of the solvent at the given temp. is derived. Its validity and general applicability have been established from experimental data on 41 oil–solvent systems. It makes data for known and unknown systems readily available in any desired units, eliminates graphical interpolations, and is applicable to all crude or refined animal, vegetable or marine oils. Experimental data are presented for a no. of systems in which commercial hexane is the solvent. J.A.C. Abstr.

Use of polythene in separation of fatty acids by reverse-phase chromatography. T. Green, F. O. Howitt and R. Preston (*Chem. & Ind.*, 1955, 591—592).—The replacement of the kieselguhr–liquid paraffin stationary phase (**I**) by powdered polythene (200 mesh B.S.) in the reverse-phase chromatographic column of Martin and Howard (*Biochem. J.*, 1950, **46**, 532) gave satisfactory results in the separation and estimation of unsaturated fatty acids with solvents of high acetone content and avoided the tendency of **I** to lose paraffin. The process works satisfactorily at room temp. for the lower acids (C_6—C_9) but a temp. of 35° is recommended for the higher acids (C_{10}—C_{20}). J. S. C.

Phospholipins. III. Chromatographic separation of glycerophospholipins. C. H. Lea, D. N. Rhodes and R. D. Stoll (*Biochem. J.*, 1955, **60**, 353—363).—Egg lecithins prepared by chromatographic separation on Al_2O_3 have low ratios of fatty acid : P, but rechromatography on silicic acid gives lysolecithin and lecithin with correct fatty acid : P ratio. Chromatography on ion-exchange columns is of little value, but with columns of silicic acid there is complete recovery of lipins and the risk of decomposition is considerably decreased. Pure phosphatidylethanolamine and lecithin are obtained from egg phospholipin by chromatography in methanol–$CHCl_3$ on silicic acid. Good separations are obtained of natural and synthetic glycerophospholipins on paper impregnated with SiO_2. J. N. Ashley.

inium in the food industry. K. Winter (*Riechst. u. Aromen*, 150—152).—The characteristics of aluminium, as pure metal, salt, are reviewed with special reference to those properties nder the material suitable for apparatus, implements, cond packaging foils used in food production. Among the cussed are: light weight, plasticity, heat-conductivity, ivity, non-toxicity, stability and indifference to chemicals n food (water, org. acids, fats, vitamins, proteins, etc.),) corrosion, which may be increased by protective coatıtural oxides (Eloxal) or inhibitors, selection of pure l or specified Al-alloys (Mg, Si, Mn) according to uitable cleaning and preservation, economical advantages: cheap production from widely distributed raw materials, easy transport, good isolating packaging material, etc. L. S.

Nutritive value of bamboo seeds (*Bambusa arundinacea* Willd.). M. V. Lakshminarayan Rao, N. Subramanian and M. Srinivasan (*Curr. Sci.*, 1955, **24**, 157—158).—The protein content of bamboo seeds is comparable with that of wheat and higher than that of rice. The nutritive value of the protein is as high as that of rice protein and higher than that of wheat protein. The complete replacement of rice in a poor rice diet by the seeds increases the growth-promoting value by approx. 50%. S. C. Jolly.

Carotene in palm oil. P. Chouard (*C. R. Acad. Agric. Fr.*, 1955, **41**, 442—446).—The occurrence, properties and behaviour of carotene are discussed with reference to the content in lucerne and carrots, and to that in the fruit of the palm, yielding palm oil. In fresh fruit the content is 30, in the oil 40—300, and in fibre oil 200—650 mg. per 100 g. Various detailed procedures for the extraction of carotene are given including extraction with methanol. Carotene from palm oil is rich in β-carotene. The rôle of carotene in animal and human foods is discussed. E. M. J.

Method of separation of the carotenoids of Caju. J. Ramos, B. M. Neto and E. de A. Melo (*Rev. Quím. industr., Rio de Janeiro*, 1955, **24**, 30).—In order to avoid the formation of artefacts, a combination of solvent partition and chromatography was applied to the separation of the carotenoids of Caju. The material was frozen, macerated and mixed with a little water when the paste had melted. This was filtered through sintered glass to remove as much water as possible and the moist paste was then extracted with a 75 : 60 mixture of acetone and hexane and filtered. The extract was washed with saturated NaCl solution to remove acetone, and then part of the hexane removed under vac. A solution of 10% KOH in methanol was then added, and the mixture agitated for 6 hr. The hexane phase was then washed with water, dried over Na_2SO_4 and evaporated. The methanol phase was extracted with chloroform and the solution was washed with water to remove soap; this was also dried with Na_2SO_4 and evaporated. Each fraction was chromatographed on alumina and the fractions checked for purity by ascending paper chromatography. H. Pritchard.

Significance of wheat germ oil in nutrition and therapy. W. Scheibe (*Dtsch. ApothZtg*, 1955, **95**, 397—398).—A brief review on the importance of wheat germ oil as vital nutrient, covering methods of obtaining the oil from the wheat embryo. The essential ingredients of wheat germ oil (vitamin E, vitamin F-factors, carotenoids and sterols), their action in the human body and their application in modern dietetics and therapy are discussed. (11 references.) J.A.C. Abstr.

Quantitative determination of calcium pantothenate, calcium gluconate, riboflavin, sorbitol and choline preparations. H. Wojahn and L. Kraft (*Dtsch. ApothZtg*, 1955, **95**, 443—444).—The estimation of Ca in Ca pantothenate and Ca gluconate is determined by the back-titration of an excess of 0·1M-Na_2 ethylenediaminetetra-acetate with 0·1M-$ZnSO_4$ in presence of a buffer at pH 8—10·0, using Eriochrome Black T as an indicator. For Ca pantothenate the method gives 98·65—98·91% of the theoretical result, and with Ca gluconate 8·92—8·94% Ca (calc. 8·94%). Riboflavin, sorbitol and Ca gluconate are estimated by keeping in the presence of 0·1N-$NaIO_4$, followed by 25% aq. $KHCO_3$ 0·1N-Na_3AsO_3 and aq. KI and the resulting solution titrated with 0·1N-I using a starch indicator. This method gives with Ca gluconate 98·8—99·75%, riboflavin 99·36—101·36%, and sorbitol 99·46—100·0% of the theoretical values. Choline chloride is determined as reineckate, followed by an addition of excess 0·1N-$AgNO_3$ and titration with 0·1N-NH_4CNS. The results are 99·6—100·2% of the theoretical. J.A.C. Abstr.

Determination of nicotinamide and nicotinic acid. I. Nicotinamide. A. Barreto, F. A. Gai and H. S. R. Barreto (*Rev. Quím. industr., Rio de Janeiro*, 1955, **24**, 12).—Chloranilic acid forms coloured ppt. with nicotinamide and nicotinic acid; these are not formed simultaneously as nicotinamides react first. The chloranilide of nicotinamide is a well-crystallised red solid, sol. in water, weakly sol. in ethanol and insol. in amyl acetate and ether. It sublimes at 180° and decomposes at 240—245°; the aq. solution has pH 3 at 23° and a conductivity of 6·0 × 10^{-4} mho/cm. The reaction was used for the determination of the vitamin either gravimetrically or colorimetrically. H. Pritchard.

Identification of vitamin C by paper chromatography of the dinitrophenylhydrazones. R. C. R. Barreto (*Rev. Quím. industr., Rio de Janeiro*, 1955, **24**, 13, 17).—During the examination of vegetable infusions for the presence of vitamin C, the ascendant–descendant paper chromatographic method of Block was used on the dinitroosazones. Many combinations of solvent were used, but a 90 : 10 mixture of *iso*butanol and 5% ammonia and a 95 : 5 mixture of

xylene and nitrobenzene were the two found most useful. The latter furnished compact spots of such definition that treatment with alcoholic KOH to reveal them was found unnecessary.
H. PRITCHARD.

Stability of ascorbic acid in various liquid media. F. J. Bandelin and J. V. Tuschhoff (*J. Amer. pharm. Ass., Sci. Edn.*, 1955, **44**, 241—244).—The rate of oxidative decomposition of ascorbic acid in aq. solutions of cellulose gums, pectin, sucrose, glucose, maize syrup and in glycerin and propylene glycol is studied, also the effect of pH on the stability in aq. solutions. Destruction of the acid is accelerated at pH >4. Addition of ethanol, polyhydric alcohols and sugars produces a stabilising effect. Syrups and high concn. of the vitamin are relatively more stable than lower concn. but this effect is not achieved by increasing the viscosity of solutions by adding gums. Vitamin B-complex factors increase the stability of ascorbic acid syrups. J.A.C. ABSTR.

Stabilisation of dehydroascorbic acid by pyrocatechol. E. Géro (*C. R. Acad. Sci., Paris*, 1955, **240**, 2176—2178).—The inhibiting action of dehydroascorbic acid (**I**) on the oxidation of *o*-diphenols (*ibid.*, 1941) is accompanied by a reciprocal stabilisation of **I**. Solutions of **I**, buffered to pH 7·4 with phosphate, were exposed for varying periods to air at room temp., and at 37°. The effects of addition of pyrocatechol (**II**) were studied and it was found that the **I** content of a solution was invariably higher, irrespective of time-interval, temp. or method of determination of **I**, in the presence of **II** than in its absence. It is suggested that **II**, or a very closely allied structure, is present in the vitamin-C_2 complex.
J. S. C.

Tetrazolium bioautography. E. Usdin, G. D. Shockman and G. Toennies (*Appl. Microbiol.*, 1954, **2**, 29—33).—Use of triphenyltetrazolium chloride for increasing the visibility of zones of bacterial growth on solid media is demonstrated, inoculum concn., tetrazolium concn., and O_2 tension being important variables. Application to the detection of folic acid with *Lactobacillus casei* is described.
E. G. BRICKELL.

Vitamin B_{12} in sewage. J. M. Whitmarsh, J. W. Albans and R. D. Wright, *Biochem. J.*, 1955, **60**, *Proc.* xxviii).—Activated, primary, and methane digester sludges contain 10 μg./g. of vitamin B_{12}. Assayable activity is extracted by boiling without autoclaving, and it is destroyed by heating at pH 11. Extracts of 40 gal. of sludge are prepared and the vitamin is adsorbed on to C or other adsorbants; in this form it could be used as a feeding stuff supplement. J. N. ASHLEY.

Food. J. R. Matchett and H. W. von Loesecke (*Analyt. Chem., Annu. Rev.*, 1955, **27**, 623—632).—A review of publications on the analysis of substances, processes, and reactions pertaining to food. It includes various analytical methods for the determination or identification of: moisture, proteins and amino-acids, metallic ions, fats and oils, enzymes, carbohydrates, vitamins, acids, colour and taste, insecticide residues, contamination and spoilage, etc., also descriptions and applications of new apparatus. (313 references.)
L. S.

Ion-exchange in foodstuffs chemistry. A. Richter (*Chem. Tech., Berlin*, 1955, **7**, 261—267).—A review of the applications of ion-exchangers, including modern resins, to controlling the composition of water for mineral water manufacture and brewing; removing various substances in wine making; preparing "humanised" and tinned milks; purification of sugar juices and solutions; purification of starch hydrolysates; removal of acid from pectin solutions; separating albuminoids and their decomposition products; and the prep. of various vitamin products. Some details of analytical procedures using ion-exchange methods are given. (28 references.)
H. L. WHITEHEAD.

Nutrient content and protein quality of quinua and cañihua, edible seed products of the Andes mountains. P. L. White, E. Alvistur, C. Días, E. Viñas, H. S. White and C. Collazos (*J. agric. Food Chem.*, 1955, **3**, 531—534).—Rat growth studies designed to test the nutritional quality of the proteins in seeds of quinua (*Chenopodium quinoa*, Willd) and cañihua (*Chenopodium pallidicaule*), cultivated, and growing wild, in the Andes mountains, together with analytical data on amino-acids and various other nutrients are presented. Results indicate that these seed products are equal or superior to common cereals with respect to nutrient and amino-acid composition, and the protein quality is equal to that of the protein of whole dried milk. (22 references.) E. M. J.

Nutrient composition of Cuban foods. I. Foods of vegetable origin. J. M. Navia, H. López, M. Cinadevilla, E. Fernández, A. Valiente, I. D. Clement and R. S. Harris (*Food Res.*, 1955, **20**, 97—113).—Data of the analyses are presented of 115 samples of foods of vegetable origin collected in Cuba between February and December 1953, the following nutrients being determined in each: moisture, ether extract, crude fibre, N, ash, Ca, P, Fe, carotene, thiamine, riboflavin, niacin, ascorbic acid, tryptophan, methionine, lysine. (33 references.) E. M. J.

Descriptive terms and points systems for rating food qualities. D. Sheppard (*Food Res.*, 1955, **20**, 114—117).—The relationship between descriptive terms in a rating scale, and the variation in interpretation by the judges are discussed. (16 references.)
E. M. J.

Nutritional adequacy of a semi-synthetic diet sterilised by steam or by cathode rays. T. D. Luckey, M. Wagner, J. A. Reyniers and F. L. Foster, jun. (*Food Res.*, 1955, **20**, 180—185).—In tests on mice fed a semi-synthetic high vitamin diet which was (*a*) non-sterile, (*b*) steam sterilised, and (*c*) cathode ray sterilised, results indicated that no consistently harmful effects were produced from steam- or cathode ray-sterilised diet fed to three full generations. General appearance, growth, and reproduction were similar in all three groups, except in one mouse of the third generation, fed the cathode-ray-sterilised diet, which developed a large tumour during lactation.
E. M. J.

Study of protein denaturation by means of the optical rotatory power. Richard Barton Simpson (*Dissert. Abstr.*, 1955, **15**, 725—726).—The denaturation of egg and serum albumins by urea and other chemical agents was studied by observation of changes in optical rotation and the kinetics of the reactions involved were discussed. J. S. C.

Photometric method for determination of proline. W. Troll and J. Lindsley (*J. biol. Chem.*, 1955, **215**, 655—660).—A specific photometric method is described for the determination of proline; it is applicable to protein hydrolysates, urine and plasma. It depends on the colour reaction given with ninhydrin. The basic amino-acids, lysine, hydroxylysine and ornithine are first removed by shaking the sample with Permutit; the filtrate is then heated with ninhydrin in acid solution and the optical density of the solution is determined spectrophotometrically at 515 mμ. Hydroxyproline gives no coloration under the conditions of the reaction with ninhydrin. Urine and deproteinised plasma are diluted ⩽20- and 10-fold, respectively, before analysis. Urine requires a prior hydrolysis with 6M-H_3PO_4 for 24 hr. to liberate proline from its conjugates or peptides. J. N. ASHLEY.

Effect of sulphur dioxide on stability of tryptophan during acid hydrolysis of proteins. Yu. N. Kremer (*Dokl. Akad. Nauk SSSR*, 1954, **98**, 627—628).—In the hydrolysis of fibrin or casein with 2—10% H_2SO_4, an atm. of SO_2 ensures considerably higher yields of tryptophan. R. TRUSCOE.

Modified method for microdetermination of citric acid. B. McArdle (*Biochem. J.*, 1955, **60**, 647—649).—A modified colorimetric method based on that of Taylor (Brit. Abstr., 1953, C, 281) is described for determination of 10—80 μg. of citrate in biological fluids. Increased sensitivity and accuracy are obtained by increasing the concn. of H_2SO_4 to 16·5N during the formation of pentabromoacetone, and by use of a solution containing thiourea, borax, and Na sulphide for the development of the final colour. Very high concn. of glucose interfere slightly; salicylic acid and other aromatic substances must be removed by a preliminary bromination.
J. N. ASHLEY.

Use of sodium hexametaphosphate in the determination of traces of lead in food. E. I. Johnson and R. D. A. Polhill (*Analyst*, 1955, **80**, 364—367).—In the method described the phosphates of the alkaline earths and Mg can be kept in solution suitably for dithizone extraction of Pb by addition of Na hexametaphosphate. Org. matter is destroyed either by wet oxidation or by ashing, the residue is dissolved in dil. HCl and the solution is boiled and filtered. The filtrate is treated with 2 ml. of 25% NH_4 citrate solution and a 10% Na hexametaphosphate solution, adjusted to pH 9—9·5 with aq. NH_3, and KCN is added (and hydroxylamine hydrochloride if much Fe is present). By a simple extraction and purification the Pb is isolated as the dithizone complex in chloroform and determined spectrophotometrically at 520 mμ. Preliminary experiments show that Bi is not so readily extracted by dithizone in presence of Na hexametaphosphate as is Pb, and interference by this element is less than in some other methods. A. O. JONOVIC

Diethylammonium diethyldithiocarbamate for the separation by chelating determination of small amounts of metals. II. The isolation determination of arsenic, antimony, and tin in organic compounds [such as foods]. P. F. Wyatt (*Analyst*, 1955, **80**, 368—
method is described for the isolation of small amounts of As, Sn in org. compounds; the sample is decomposed with co and the metals, reduced to their lower valency states, are with a solution of diethylammonium dithiocarbamate (D form. After investigation of the limited no. of metho for the subsequent determination of these elements, the recommended and described: (*a*) micro-titration c

$KBrO_3$ using 1-naphthoflavone as indicator, or absorptiometric determination by reduction of molybdoarsenate to molybdenum blue; (*b*) micro-titration of $Sb^{\cdots}$ with $KBrO_3$ (as for As) or extraction of $SbCl_5$ into *iso*propyl ether and absorptiometric determination by means of Rhodamine-B; and (*c*) determination of Sn either by reaction of Sn^{IV} with 8-hydroxyquinoline, extraction with chloroform and absorptiometric determination, or by turbidimetric determination by means of 4-hydroxy-3-nitrophenylarsonic acid. Cu and Bi interfere but can be removed by extraction with **I** before the reduction of As, Sb, and Sn to their lower valency states, since As^{V}, Sb^{V}, and Sn^{IV} do not form complexes with **I**. A. O. JONES.

Carcinogenic activity of certain dye-stuffs. Importance in alimentary hygiene. II. R. Trubhaut (*Ann. pharm. franç.*, 1955, **13**, 87—111).—The importance of differing effects on different species of animal and of the cumulative effect of very small doses of carcinogenic compounds and of diet upon the incidence of cancer and the delay in its appearance is discussed. The various methods employed or advocated in different countries to control the use of injurious colouring matters are discussed. Appendices give a summary of French law on the subject and provisional lists of acceptable synthetic colouring matters for foodstuffs published by various committees. (85 references.) E. J. H. BIRCH.

Identification of spice-extract preparations. E. Benk (*Riechst. u. Aromen*, 1955, **5**, 114).—A review dealing with the legal (German) aspects concerning the composition of various marketable spice extracts (pepper, paprika, nutmeg, etc.) containing glucose as carrier substance, their hygienic (germ-free) packing and differentiation between natural spices, extracts, and substitutes. L. S.

Extraction of colouring agents for foodstuffs with quinoline and their identification by chromatography on aluminium oxide "plates." M. Mottier and M. Potterat (*Anal. chim. Acta*, 1955, **13**, 46—56).—A rapid scheme for separating and identifying artificial colouring matter from foodstuffs is described. Water-sol. dyes are extracted with quinoline from an aq. solution or suspension of the sample buffered at pH 3. Fat-sol. dyes are adsorbed directly on to the activated Al_2O_3 from a solution of the sample in a solvent of weak eluting power. The dyes are then separated chromatographically on Al_2O_3, and the results are compared with known dyes submitted to the same techniques. If required, the chromatograms are preserved by eluting with molten paraffin wax or cetyl alcohol and allowing to dry. J. H. WATON.

Assay method for papain using a synthetic substrate. L. B. Stadler (*Dissert. Abstr.*, 1955, **15**, 714—715).—A new assay method for papain is based on its ability to liberate NH_3 from a synthetic substrate, by allowing it to act on an acylated amino-acid and measuring the NH_3 formed by Nessler's reagent. The most satisfactory substrate proved to be hippuryl amide. A considerable no. of tests was made on a wide variety of samples. J. S. C.

Comparative sensitivity of rating scale and paired comparison methods for measuring consumer preference. F. J. Pilgrim and K. R. Wood (*Food Technol.*, 1955, **9**, 385—387).—In tests the rating scale and paired preference methods were equally sensitive whether the difference in preference was small or large. (12 references.) E. M. J.

A taste panel study of Cyclamate–saccharin mixture and of its components. H. C. Vincent, M. J. Lynch, F. M. Pohley, F. J. Helgren and F. J. Kirchmeyer (*J. Amer. pharm. Ass., Sci. Edn*, 1955, **44**, 442—446).—Studies on the off-taste and relative sweetness of 10 : 1 mixtures of Cyclamate (Na *cyclo*hexylsulphamate) and saccharin in solution, using the taste panel technique, show that this mixture is sweeter than would be expected from the individual components, while the off-taste is minimised. Cyclamate-Na is 30 times as sweet as sucrose at a concn. of 0·17%, dropping to 15 times at 0·36%. In both compounds the Na salt is sweeter than the Ca salt. Twenty % of the population are expected to detect the off-taste of 0·53% of Cyclamate-Na and 0·053% of saccharin-Na. G. R. WHALLEY.

"aste panel study of the saccharin "off-taste." F. J. Helgren, Lynch and F. J. Kirchmeyer (*J. Amer. pharm. Ass., Sci. Edn*, **44**, 353—355).—Using unheated and freshly prepared saccharin ns, a panel of tasters studied the incidence of off-taste. Trace ies, thermal decomposition, and age of solution are not ' for the off-taste, and approx. 25% of the populace can be to detect it at a saccharin concn. up to 0·026% (≡10% nd approx. 50% at 0·07% (≡28% sucrose). The off-taste the saccharin molecule itself, and may be detected by ıdividuals. G. R. WHALLEY.

nonwealth as a source of essential oils. M. F. Carroll . *Arts*, 1955, **103**, 689—701).—Sections deal with: th production in comparison with world production of economic value of the principal oils and their uses, various processing methods, extraction or synthesis of isolates, factors affecting production and sale of essential oils, the future of the industry, and the potentialities of Commonwealth sources. G. HELMS.

Spectrophotometric analysis of the essential oils of citrus fruits. R. Cultreva and E. Trifirò (*Chim. e Industr.*, 1955, **37**, 701—705).—Previous methods (E. Trifirò *et al.*, *Conserve e Derivate Agramari*, 1952, **1**, 2, 18; 1953 **2**, 77; 1954, **3**, 5) are further developed. Data are obtained which make possible a rapid estimation of the purity of an essence, and show the presence of adulterating matters. A series of transmission curves is illustrated for various types of essences. C. A. FINCH.

Classification of essential oils by means of the straight line relationship between index of refraction and specific gravity which is a guide to the chemical composition. L. H. Narodny (*Perfum. essent. Oil Rec.*, 1955, **46**, 145—146).—Data on oils from widely different sources show that the formula $n^{20} = 0{\cdot}51\ d_{15}^{15} + 1{\cdot}019$ may be applied to all citronella oils between the limits 0·8785 and 0·8955 for *d*. It affords a valuable tool at the point of production, to determine the length of time a distillation should be allowed to run before % of citronella falls too low. It is suggested that a similar relationship between *n* and *d* may be found for almost all essential oils. J.A.C. ABSTR.

Essential oils and related products. E. Guenther and E. E. Langenau (*Analyt. Chem., Annu. Rev.*, 1955, **27**, 672—677).—A review on literature on analytical procedures applied to essential oils and allied substances, covering the period Oct. 1952 to Oct. 1954. Apart from products, specifications, and test methods published by the B.P. 1953, the Syndicat National des Fabricants et Importateurs d'Huiles Essentielles et Produits Aromatiques Naturels of Grasse 1954, the British Standards Institution 1954, the Scientific Committee of the Essential Oil Assoc., U.S.A., and other official compendia, a number of papers are discussed dealing with absorptiometric, paper-chromatographic, and other analytical procedures for the determination of acids, alcohols, phenols, esters, aldehydes, ketones, terpenes, peroxides, essential oil contents in drugs and spices, water contents in drugs and solvents, etc. (135 references.) L. S.

Microbiological spoilage of canned vegetables and fruits. I. W. J. Hoppenbrouwers (*Conserva*, 1955, **3**, 362—366).—A review covering pH–temp. relationships in heat-sterilising, and characteristics of thermophilic and mesophilic spoilage bacteria. P. S. ARUP.

Microbiological detection of preservatives in foods. M. J. Bernaerts (*Conserva*, 1955, **4**, 38—42).—Available techniques and their applications in testing different foods are briefly described, and their advantages and limitations are discussed. Standardisation of procedures is recommended. P. S. ARUP.

Preservation of foods. VII. Preservation by heating. H. A. Leniger (*Conserva*, 1955, **3**, 370—376).—A review covering the physics of heat-transfer as applied to food sterilisation. P. S. ARUP.

Preservation of foods. VII. Preservation by heating. vi. J. A. Glerum and H. A. Leniger (*Conserva*, 1955, **4**, 49—56).—A review covering practical directions and descriptions of autoclaves and steamers. P. S. ARUP.

Effect of storage on the ultra-violet absorption of fresh and stored hen's eggs. W. Rauch and G. Varela (*An. Bromatologia*, 1955, **7**, 13—23).—The white of egg in 1% aq. solution shows a rising absorption at 280 mμ. on storage; e.g., storage for 60 days raises the u.v. absorption coeff. from 0·9 to 1·27, when dehydration has been allowed for. L. G. L. UNSTEAD-JOSS.

Vapour pressure requirements for cold storage structures. S. J. Stachelek (*Industr. Refrig.*, 1955, **128**, No. 6, 34, 36—38).—The design of cold storage units and the selection of wall materials with suitable vapour permeance are discussed. A table of permeance values for a wide range of building materials is given. J. S. C.

Control of relative humidity in cold stores. G. Mann (*Proc. Inst. Refrig.*, 1953—54, **50**, 177—214).—Experiments were carried out to obtain data concerning the effects of variations in cooler area, air speed, heat leakage, and coolant temp., on wt.-loss by evaporation of potatoes both during the cooling-down period and the subsequent steady state. The most important factor controlling humidity was the cooler area. During cooling, the wt.-loss was found to be independent of coolant temp. With full cooler surface being used and max. air circulation of 65 charges/hr., the wt.-loss was 0·55% of produce wt. In steady-state conditions, the wt.-loss was found to be greater with intermittent cooling with coolant at 20°F. than with continuous cooling with coolant at 34°F. J. S. C.

Refrigerator acting as heat pump: application to the preservation of foods (grain, sugar, etc.). Creuzot and Leroy (*Industr. agric.*

aliment., 1955, **72**, 403—405).—The preservation of foodstuffs by freeze-drying is discussed theoretically. By recirculating the air from the drying chamber to the condenser of the refrigerator, it can be made to absorb more heat than was extracted from it in the drying process, and the refrigerator acts in effect as a heat pump. J. S. C.

The work of European refrigeration laboratories and its industrial applications. M. Anguez (*Rev. gén. Froid*, 1955, **22**, 783—787).—An account is given of the findings of an O.E.E.C. team which visited plants for quick-freezing of meat at Pforzheim, Germany, fruit preservation of the East Kent Packers' Co-operative at Faversham, and quick-freezing of fish at Bergen. J. S. C.

Problem of seed-grain storage in connexion with [use of] the combined harvester and thresher. E. Bernfus (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 95—99).—A review covering directions for the adequate ventilation and drying of the grain during storage in order to prevent deterioration by infection with spoilage micro-organisms or other causes. P. S. ARUP.

Effects on maize of storage in airtight bins. G. H. Foster, H. A. Kaler and R. L. Whistler (*J. agric. Food Chem.*, 1955, **3**, 682—686).—Observations were made on eight lots of shelled maize in test bins during the period 1949–52. Odour and colour changes occurred which were augmented by high moisture contents, longer storage periods, and higher grain temp. Maize containing 18% of moisture after storage for one year darkened slightly and developed a sour odour, while maize containing 23 or 27% of moisture developed a sour odour in less than a month, and a darker colour. There was increase in fat acidity and in no. of damaged kernels towards the end of storage for one year; the maize became very difficult to handle, and rapidly became mouldy on removal from the bin. Pigs fed maize containing 27% moisture stored for two seasons, remained healthy and made normal wt. gains. E. M. J.

Recovery of fat from animal fat-containing products. British Glues and Chemicals, Ltd. (Inventor: I. H. Chayen) (B.P. 722,311, 5.8.49. Addn. to B.P. 714,671, 25.5.50).—The fat-containing meat material is treated with cold water (at least three times the wt. of the fat) in a swing-beater type hammer mill, to break the membranes and release the fat, which passes a 200-mesh sieve as a foam into a settling tank from which an aq. and a fatty layer are separately removed. J.A.C. ABSTR.

Continuous hydrogenation of fatty oils and fatty acids. Bamag, Ltd., and B. Bregman (B.P. 723,887, 20.9.51).—Fatty material is continuously hydrogenated by means of an intimate and vigorous contact therewith of a catalyst and H_2; a mixture of the fatty material and catalyst is caused to flow in a laterally confined stream having its upper surface continuously exposed, and H_2 is introduced throughout the length of the flowing stream in jets whereby the stream is continuously agitated to maintain distribution of the catalyst in the fatty material. The jets may be arranged to change direction cyclically. Excess H_2 is withdrawn continuously from the upper surface of the stream. J.A.C. ABSTR.

Gas-tight containers for the preservation of liquids. G. M. Jennings (B.P. 721,277, 10.8.51).—The gas-tight container has a screwed plug in its neck, which houses a valve responding to 10 atm. pressure. The valve has a central spindle carrying a cup-shaped member with a resilient filling. A spring presses against the member and when it is compressed flow of the fluid is allowed. The valve is covered with a cap and the plug opening sealed. Withdrawal of the liquid is carried out by a plunger-tap type of apparatus. In this container liquids, e.g., milk, may be maintained in an atm. of, e.g., oxygen, under pressure. J.A.C. ABSTR.

Apparatus for recovering waste pickling solutions. Soc. O. Fakler & Adam (B.P. 722,832, 7.4.52. Fr., 26.4.51).—Stirrers in the crystallising tank prevent accumulation of crystals on the walls, and impart rotatory motion to the body of the fluid. Liquid moving with the greatest peripheral velocity is removed tangentially under the action of pressure caused by the centrifugal forces and flows into a reservoir at the same level. The crystals settle out and the liquid is returned to the pickling vat via the crystallising tank. (Cf. B.P. 655,799.) J.A.C. ABSTR.

3.—SANITATION

Modified "pyrethrin II" assay. W. Mitchell and F. H. Tresadern (*J. Sci. Food Agric.*, 1955, **6**, 465—467).—It is recommended that chrysanthemumdicarboxylic acid being readily sol. in boiling water, should be separated from water-insol. acidic matter, before titration, in the determination of "pyrethrin II" by the A.O.A.C. (or Seil) method. The accuracy of the determination is improved. E. M. J.

Determination of calcium and magnesium by means of sodium ethylenediaminetetra-acetic acid [Complexone III], especially in mineral waters. K.-E. Quentin (*Z. LebensmittUntersuch.*, 1955, **102**, 106—117).—Conditions affecting the determination of Ca by titration with Complexone III, using murexide, and of (Ca + Mg), using Eriochrome black T as indicator, are examined, and directions are given for the prep. and standardisation (where necessary) of the necessary solutions and the murexide indicator mixture, and for the carrying out of titrations. Amounts of Al, Mn, Fe and Cu in excess of 10, 2, 3 and 0·6 mg. per l., respectively, interfere with the titrations, and must be removed by the addition of Na diethyldithiocarbamate and shaking out with chloroform. By following the directions given, results of satisfactory accuracy can be obtained within 1 hr. (42 references.) P. S. ARUP.

Determination of substances in minute quantity. X. Colorimetric determination of sodium in water. T. Kato, Y. Okinaka and T. Nomura (*Technol. Rep. Tohoku Univ.*, 1954, **19**, 81—84).—Na may be determined in water by pptg. sodium magnesium uranyl acetate, dissolving it in hot water and determining the Mg. colorimetrically using 0·1% Titan Yellow. KCl interferes; NH_4 sulphate, Ca nitrate and ferric alum cause no interference. J.A.C. ABSTR.

Molybdenum blue reaction and determination of phosphorus in waters containing arsenic, silicon and germanium. H. Levine, J. J. Rowe and F. S. Grimaldi (*Analyt. Chem.*, 1955, **27**, 258—262).—Factors influencing the determination of P in presence of As, Si and Ge by the molybdenum blue method (absorptiometric) are examined and a new procedure recommended for sea waters. This involves co-precipitation of the phosphate with $Al(OH)_3$ and the determination of the PO_4''' in the ppt. J.A.C. ABSTR.

Water works practices: instrumentation and control. G. E. Symons (*Wat. & Sewage Wks*, 1955, **102**, 203—213).—The subject is systematically and fully reviewed under the headings: definitions, reasons for instrumentation, reasons for control, measured variables, points of use, primary devices, transmission, secondary instruments, selection of instruments, control considerations, plant control, control of levels, pressure pumps, chemical feed, temp. and humidity, supervision and control panels. J.A.C. ABSTR.

Anaerobic contact process as applied to packinghouse wastes. G. J. Schroepfer, W. J. Fullen, A. S. Johnson, N. R. Ziemke and J. J. Anderson (*Sewage industr. Wastes*, 1955, **27**, 460—486).—A description is given of the investigation on pilot plant scale of the period of four years. The pilot plant is described in detail and consists of holding tank, digester, evacuator tank, separator tank and trickling filter with the usual ancillary apparatus. The solid particles were found to be of a low sp. gr. and a settling additive (fly ash) was used to enable separation to take place more quickly. One test programme was primarily planned to consider the effects of a no. of variables taken individually such as loading vac. temp. and degree of mixing. Supplementary investigations covered the power requirements, the dewatering of sludge on vac. filters, a chlorine demand and trickling filter efficiency. A comparison with aerobic conditions is given. J.A.C. ABSTR.

Some residual effluent problems. F. W. Roberts (*J. Inst. Sewage Purif.*, 1954, 31—37).—Factors influencing the brown coloration of sewage effluent are discussed, particularly in relation to the effect of spent gas liquors. Slight increases of pH between 7 and 8 increased coloration by gas liquors in oxidising (aeration) conditions. Colour formation was greatly reduced by extraction with butyl acetate or methyl *iso*butyl ketone. Tests to determine the toxicity of anionic detergents likely to be found in sewage were carried out on rainbow trout, fresh water shrimps (*Gammarus pulex*), and pond weed (*Potamogeton densus*). Residues of detergents in effluent are not modified in toxicity by passage through an efficient sewage purification process and concn. present in an effluent are liable to be of the same order as those found toxic in short-term laboratory trials, although their effects may be modified by the ability of organisms to become acclimatised. J.A.C. ABSTR.

Purification of waste waters in plating plants. S. Ziva (*Zastita Materij.*, 1955, **3**, 87—92).—The necessity of tail- clarification and recovery of material from wastes of electro- operations is pointed out. Methods of removing cyanides, ates, injurious metals and acids are discussed and the pro- neutralisation, purification and regeneration of waste lic explained with the aid of a flow diagram. J.A.C.

Gas liquor problem. A. D. Burman (*J. Inst. Sewage P.* 69—74).—The problems of disposal of gas works effl- viewed historically and various methods of treatment ar- including the steam distillation of crude liquor, solve- with tar oil and with butyl acetate (Phenosolvan), ammonia-still effluent with HNO_3, ion-exchange me-

and cold electro-detarring. Some laboratory experiments on ion-exchange techniques, using Decolorite and Deacidite FF resins, are also described. J.A.C. ABSTR.

Ultimate disposal of radioactive wastes. W. S. Ginell, J. J. Martin and L. P. Hatch (*Nucleonics*, 1954, **12**, No. 12, 14—18).—Investigations of the absorption of radioactive cations in a montmorillonite clay, using ^{90}Sr, ^{91}Y, ^{95}Zr, ^{139}Cs, ^{144}Ce, ^{147}Pm and mixed fission products as tracers, are described. The exchanged active cations are fixed in the crystal lattice by heating at 900° or above. After heat treatment, leaching by sea water or other suitable replacement solution removes only a very small proportion of the activity from the clay. SCI. ABSTR. J.A.C. ABSTR.

Radioactive-strontium [contamination] removal by lime-soda softening. R. F. McCauley and R. Eliassen (*J. Amer. Wat. Wks Ass.*, 1955, **47**, 494—502).—^{90}Sr ions can be co-pptd. with $CaCO_3$ to form calcite–strontianite mixed crystals in the cold lime–soda process. In the hot lime–soda process the ppt. is aragonite–strontianite. Ca hardness must be reduced to the point of equilibrium to secure max. removal of ^{90}Sr. One pass through a softener operating at max. efficiency effects 50% removal and repeated pptn. of $CaCO_3$ will remove more than 99% ^{90}Sr. The presence of up to 10 p.p.m. of stable Sr isotopes does not interfere with the removal of ^{90}Sr. The adaptation of conventional water treatment plants for this purpose is described. J.A.C. ABSTR.

Determination of radioactive strontium and barium in water. R. B. Hahn and C. P. Straub (*J. Amer. Wat. Wks Ass.*, 1955, **47**, 335—340).—A method is described by which low concentrations of radioactive Sr and Ba (discharged into water by nuclear power industries) can be determined with ~10% precision, after evaluation of interfering substances (Ca, Mg, Fe, etc.) and suitable concentration of radioactive components. Methods of concentration (by evaporation, ion-exchange or precipitation) are discussed and a detailed analytical technique in 18 stages is appended. Tests with tracer solutions of known activity showed the method to be sensitive for Sr concn. of 4×10^{-8} and Ba concn. of 10^{-7} μc./ml. Different isotopes (e.g., ^{89}Sr and ^{90}Sr, ^{137}Ba and ^{140}Ba) cannot be distinguished by the process described and would require further decay- and absorption-analysis. J.A.C. ABSTR.

Treatment of municipal wastes by composting. H. C. Husband (*Munic. Util.*, 1954, **92**, No. 7, 31—34, 56—60).—Composting may be used in the treatment of sewage sludge. Investigations were made to find a method of decomposting org. wastes hygienically, quickly, and with a min. loss of valuable constituents. For the best conditions of decomposition, the initial C : N ratio should be between 30 and 40 to 1 and the initial moisture content between 45 and 65% of the total wt. It is advisable to break up the wastes into small particles, but not so small that they compact and give rise to anaerobic conditions. The initial pH of the mixture should be between 6 and 7. Precautions should be taken against flies breeding in composts out of doors. Particularly in countries with a climate such as Canada, suitable conditions for composting must be provided artificially. To renew the supply of O_2 the compost is periodically stirred and information is given about when to turn it. Stirring is also a means of controlling temp. Usually the process is most economical when garbage and sewage sludge are composted together. The height of the bed should not exceed about 5½ ft. WAT. POLLUT. ABSTR. J.A.C. ABSTR.

Relation of coliform-organism test to enteric-virus pollution. F. W. Gilcreas and S. M. Kelly (*J. Amer. Wat. Wks Ass.*, 1955, **47**, 683—694).—Laboratory studies were carried out to determine whether the conventional coliform tests are a valid indication of enteric-virus pollution in water. It is concluded that, in general, they are and that they are also useful in evaluation of anti-pollution treatments. (12 references.) J. S. C.

Sources of air pollution literature. J. B. Murk (*Industr. Engng Chem.*, 1955, **47**, 976—981).—A bibliography of bibliographies on air pollution has been prepared. Legislation, institutional work, symposia are cited. Editorial problems are discussed. Lists given of publications containing articles and abstracts on air tion, and of organisations working on the subject. (105 nces to literature, 34 to publications, 33 to organisations.) J.A.C. ABSTR.

APPARATUS AND UNCLASSIFIED

ıl method with sealed tube digestion. Factors influencing decomposition. B. W. Grunbaum, P. L. Kirk, L. G. C. W. Koch (*Analyt. Chem.*, 1955, **27**, 384—388).—A nade of the factors influencing ammonia decomposition d-tube Kjeldahl digestion procedure. The NH_3 loss on is due to its oxidation to N_2 gas and may be caused by decomposition of NH_4HSO_4 or oxidation of NH_3 by SO_3 or by O_2, at temp. >500°. The quantity of H_2SO_4 used and prolonged digestion times may also be responsible for considerable NH_3 loss. The data obtained indicate that digestion times of 0·5 hr. are more than adequate for most organic compounds and that addition of a little water to the digestion mixtures markedly increases the stability of NH_3 in H_2SO_4. J.A.C. ABSTR.

High rate of shear rotational viscometer. E. M. Barber, J. R. Muenger and F. J. Villforth, jun. (*Analyt. Chem.*, 1955, **27**, 425—429).—The design is given of a rotational viscometer for studying the behaviour of non-Newtonian liquids up to a shear rate of 10^6 reciprocal sec. The heating effect due to the high rate of shear is controlled by employing thin films of the test liquid, and by arranging for an equal heat path through the inner and outer cylinders which are maintained at the same temp. Data are given for several mineral oil–polymer blends. Good agreement is found with data already published for two of these mixtures, where different apparatus and techniques were employed. No permanent viscosity change is detected after prolonged and high rates of shear. J.A.C. ABSTR.

Separation of substances by means of high voltage paper-electrophoresis. G. Werner and O. Westphal (*Angew. Chem.*, 1955, **67**, 251—256).—A process for paper electrophoresis is described in which voltages of up to 10,000 v. and migration passages of up to 1 m. can be used. The strip of paper is moistened with the solution of the substances to be separated by a method which permits uniform and accurate moistening, and the treated paper strip is subjected to the charge in a " moist chamber " while lying on a synthetic resin plate cooled on the underside by a circulating fluid which keeps the paper temp. <0°. Condensation of water on to the paper is avoided. This method avoids the difficulties encountered in direct cooling of the paper with cooling liquids. A description is given of the use of the process for the separation of mixtures of amino-acids, e.g., mixtures of glycine, alanine, α-aminobutyric acid, serine, threonine, methionine, glutamic acid, asparaginic acid, oxypyrroline, dihydroxyphenylalanine; mixtures of peptides; mixtures of sugars, e.g., of glucose, mannose and rhamnose; and mixtures of inorg. ions (Fe^{II}, Co^{II}, Cd, Pb^{II}, Cu^{II} and Fe^{III}). (32 references.) J.A.C. ABSTR.

Statistical comparison of three methods for determining organic peroxides. C. Ricciuti, J. E. Coleman and C. O. Willits (*Analyt. Chem.*, 1955, **27**, 405—407).—The three methods compared were the polarographic procedure of Willits *et al.* (*ibid.*, 1952, **24**, 785), the $SnCl_2$ procedure modified by Barnard and Hargrave (*Anal. chim. Acta*, 1951, **5**, 476) and the Wheeler iodide procedure (*Oil & Soap*, 1932, **9**, 89). With pure hydroperoxides the three methods are comparable, but with impure products the polarographic technique may be more reliable. For some samples the chemical methods gave values which were significantly higher than those by the polarographic method. J.A.C. ABSTR.

Application of isotope derivative method to analysis of pyrimidines. J. R. Fresco and R. C. Warner (*J. biol. Chem.*, 1955, **215**, 751—763).—A scheme is given for the analysis of μg. amounts of uracil and thymine. These pyrimidines are converted by *p*-iodobenzenesulphonyl chloride (" labelled " with ^{131}I or ^{35}S) in 75% acetone buffered with tetramethylammonium bicarbonate to pH 8·5—9·0, at room temp. into the corresponding sulphonyl deriv., which are then determined by carrier or indicator methods, using standard techniques. An advantage of the isotope deriv. method is that quant. isolation of the deriv. is not needed. The method is applicable to determination of uracil and thymine in protein hydrolysates. J. N. ASHLEY.

Determination of amides in aqueous and non-aqueous solution by the Conway diffusion technique. H. E. Hallam (*Analyst*, 1955, **80**, 552—556).—A rapid method is described for the determination of amide N with an accuracy of ± 0·2%. The base is liberated by 1 ml. of 10N-KOH and is absorbed in 1 ml. of 1·5% H_3BO_3 containing a methyl red–bromocresol green indicator. The absorbent is finally micro-titrated with 0·02N-HCl. Formamide can be determined in 3 hr. and acetamide in 5 hr.; these times are considerably reduced with oscillation on a Gallenkamp oscillation table. *N*-Methylacetamide needs 32 hr., owing to the very slow absorption of methylamine. With urethane, the rate of hydrolysis is too slow for application of the method. With non-aq. solutions, difficulties occur owing to condensation and creeping of the solvent, and duplication of the normal no. of three units per determination is necessary to allow for rejection of ruined determinations. Determination of *N*-methylacetamide in CCl_4 is apparently impossible, all units being contaminated with solvent and alkali in the long absorption period. Amides that cannot be determined directly by the method require the normal Kjeldahl digestion beforehand. A. O. JONES.

SOCIETY OF CHEMICAL INDUSTRY

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J. Sci. Food Agric., 6, December, 1955

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

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